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The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources

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The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources

Proceedings of International Training Workshop

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Food & Fertilizer Technology Center for the Asian & Pacific Region (FFTC)
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Taiwan Agricultural Research Institute (TARI)

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Preface

The “International Training Workshop on the Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources,” sponsored by the Council of Agriculture (COA), Taiwan and the Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC), was successfully held on January 9-11, 2007. The Workshop was hosted by the Taiwan Agricultural Research Institute (TARI) and taken place at the Southern Fruit Research Institute (SOFRI) at My Tho, Tien Giang, Viet Nam.

Plant genetic resources (PGR) are the basis of crop improvement. Genetic variations and diversity of plant are the motivation force for continuous progress in breeding. In recent years, agricultural production and management have adopted various elite cultivars and a more specialized economic production model. The demand of new agricultural products is on the increase in almost all the countries due to population increase so that PGR is the basis for developing, producing and marketing of new food, healthcare and pharmaceutical products. In the tropical and subtropical countries of the South Asian and Pacific region, there are abundant PGRs which require good practices of genebank management. In other words, the conservation, development and sustainable utilization of PGR should further be improved. An alarmingly high number of stored accessions are in need of regeneration as a significant proportion of the materials collected in the past is now under the endangered situation. Therefore, it is very meaningful to hold this Training Workshop so as to promote the collaboration on the exchange and sharing of plant germplasm materials and practice experiences.

The Training Workshop focused predominantly on the conservation and utilization of tropical/subtropical PGRs. The major topics included (1) the information, knowledge, and practices regarding the conservation and utilization on PGRs; (2) new technology and efficient management of PGRs; (3) particular problems, constraints and solutions of the conservation and utilization of PGR; (4) decisions on appropriate tropical/subtropical plant germplasm management strategies.

The delegates, researchers or experts on PGR from Indonesia, Malaysia, Myanmar, Philippines, Taiwan, Thailand, and Vietnam were invited to attend and requested to deliver technical or country reports in the Workshop. More than 10 PGR coordinators or experts from research institutes, universities, and related agencies in Vietnam submitted scientific posters for exhibition in the Workshop. This proceedings cover all the scientific papers presented by the invited speakers, which should be of significant value to the agricultural scientists and industries dealing with the conservation, utilization, and management of PGRs.

I would like to take this opportunity to express my gratitude to all participants whose attendance contributed to the very success of the Training Workshop. Special thanks are given to all the speakers for their prompt preparation of manuscripts and presentation materials. The Workshop and Proceedings would not have been possible without the financial support from the Council of Agriculture (COA), the superior assistance from FFTC, SOFRI, and the excellent assistance from the staff members of SOFRI.

Chien-Yih Lin, Ph.D.

Director General
Taiwan Agricultural Research Institute
Council of Agriculture

Welcome Address

Dr. Nguyen Minh Chau

Director, Southern Fruit Research Institute (SOFRI)

Tien Giang – Viet Nam

Respected Professor Hong-Ji Su, Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC); Dr. Sheng-Chung Huang, Director of National Plant Genetic Resources Center, Taiwan Agriculture Research Institute (TARI); Mr. Nguyen Van Khang, Director of Service of Agriculture and Rural Development of Tien Giang Province. Ladies and Gentlemen,

It is my great pleasure to welcome all of you on behalf of all the staff of the Southern Fruit Research Institute (SOFRI). I wish all of you in good health and having very good time in Vietnam.

Dear friends,
Plant Genetic Resources are very important to us, as with the intensive agriculture those valuable resources were and being disappeared. Hence, a joint efforts and actions of all of us to save those resources are very necessary before these plant resources disappeared, particularly those of medicine plants, and disease resistant genes in wild plants.

I am happy that the Taiwan Agriculture Research Institute (TARI), and the Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC) have decided to start their efforts in saving those plants with this workshop. I think we all hope this workshop will soon lead to

a project which aiming at collection, conservation, evaluation and utilization in improving our crop plants. With a joint efforts, we can share our methodology in screening those valuable resources against insect and diseases, and donate those valuable resources to others international centers or countries who can conserve them before they are disappeared. As a result, we can know these plants characteristics faster than doing alone in each country.

Dear friends,

FFTC has many experiences in running international projects like the citrus greening, and improving banana projects for some Southeast Asian countries, Vietnam had been selected by FFTC to cooperate with since 1996. Southern Fruit Research Institute has started to work with FFTC, Taiwan University, Taiwan Banana Res. Institute since 1996. With my experiences with FFTC, I strongly believe that with new project, together we will be able to save lot resources and be able to screen our plants resources for diseases resistant faster.

Last but not the least; I would like to thank FFTC who has chosen Southern Fruit Research Institute as the co-organizer of this important meeting and to thank to all the speakers for coming. I would like all of you have a pleasant an enjoy stay in Vietnam. If you need any support from the local co-organizer, please do not hesitate to talk to us.

For the study tour, Dr Sheng-Chung Huang and I would like to show you not only the Mekong Delta but also the highland of South Eastern part where we have a lot of rubber trees, durian, jackfruit and rambutan trees..…

Thank you.

Opening Address

Dr. Chien-Yih Lin

Director General, Taiwan Agricultural Research Institute, COA

(Addressed by: Dr. Sheng-Chung Huang, Director, PGRC, TARI)

Honorable Director Nguyen Van Khang, Service of Agriculture and Rural Development; Ms. Nguyen Thi Thanh Mai, Principal Specialist, Department of Agronomy – MARD; Dr. Nguyen Minh Chau, Director, Southern Fruit Research Institute (SOFRI); Dr. Hong-Ji Su, FFTC representative, distinguished speakers, honorable guests, dear participants, ladies and gentlemen:

First of all, on behalf of one of the Committee Organizers, the Taiwan Agricultural Research Institute (TARI), I would like to thank FFTC and SOFRI for taking part in co-organizing this important workshop. Please also accept my sincere apology for not being able to be present at this event because of prior commitments. I would also like to express my sincere appreciation to Dr. Chau and his colleagues for holding this timely and meaningful International Training Workshop on "The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources".

The importance of plant genetic resources has been well recognized worldwide. The greatness of agricultural improvement depends on not only the advancement of scientific research but also the utilization of abundant and diversified plant genetic resources. The resulted improvement in both the yield and quality of agricultural products has exerted impacts on almost every aspect of our life.

Due to the ideal geographical location, Taiwan has been benefited by the abundance of tropical and subtropical plant genetic resources in the development of agricultural industry. We also have established core facilities of gene bank and integrated information system of management. As one of the Southeast Asian countries, we realize the importance of multilateral cooperation of exchanging and sharing technology and materials on the collection, identification, and conservation of plant genetic resources. We believe that the continuous advancement of agricultural production in the whole Southeast Asian region can be achieved only through such kind of cooperation. During the past decades, Taiwan has been cooperating with many neighboring countries in this aspect. One very significant example is that by sending agricultural missions abroad, our specialists offer and share our learning and experiences in plant genetic resources with local scientists, farmers, and agricultural industries.

In this workshop, we are honored and pleased to invite distinguished speakers from Indonesia, Kampuchea, Malaysia, Myanmar, Philippines, Taiwan, Thailand, and Vietnam to present their specialties in plant genetic resources. In addition, many experts of Vietnam contribute posters for exhibition and offer their experiences and expectations to a new and more advanced agricultural production. On this special occasion, I foresee opportunities of international cooperation in the resolution of general and particular problems and constraints in gene bank management and utilization of germplasm.

Finally, I wish for every success of this international training workshop. I also wish to extend my sincerest welcome and blessings to all of you. To our friends, I wish all of you a very pleasant stay in this lovely and friendly country – Viet Nam.

Thank you very much.

Special Remarks

Mr. Sing-Hwa Hu

Director, Food and Fertilizer Technology Center (FFTC)

(Delivered by: Dr. Hong-Ji Su, Professor Emeritus, Dept. of Plant Pathology, National Taiwan University/Consultant, FFTC)

Dear Participants and Distinguished Guests:

On behalf of FFTC, it is such a great pleasure for me to express my warmest welcome to all the participants from eight countries of Southeast Asia, attending this international workshop on Conservation and Utilization of Tropical and Subtropical Plant Genetic Resources. While our FFTC Director, Mr. Hu, is unable to join us in this activity due to a very important prior commitment, I would like to convey on his behalf our heartfelt gratitude to the organizers, especially to Dr. Chau, Director of SOFRI, and co-organizers Dr. Hong also of SOFRI and Dr. Huang, Director of the Taiwan National Genebank, TARI. Without their utmost efforts, the success and smooth conduct of this present workshop would not have been possible.

The primary goal of this workshop is to promote the full utilization and effective conservation of plant genetic resources through international exchange of new information, technical experience, and useful germplasm exchange. We are sure that these activities will be much beneficial to each participating country in Southeast Asia.

Through the full utilization of genetic resources, agro-industries will be able to better produce enough foods and grow better living environments for the people. Likewise, in the face of a looming global energy crisis and the depletion of energy sources such as oil/gas, bioenergy from plant genetic resources (PGR) will be our only hope for the future. Bioenergy can be generated from PGR via the photosynthesis of plants from the solar energy. Most importantly, PGR is the basic foundation of crop improvement. Genetic variation and diversity of plants are the motivating force for the continuous progress in breeding and crop

improvement, in particular, and in the attainment of sustainable agriculture in general.

We deeply congratulate all the scientists and experts in the area of plant genetic resources, for your valuable contributions to this scientific field. We likewise encourage each and every one of you to continue this scientific and moral mission to preserve and conserve, as well as to efficiently and fully utilize the plant genetic resources of the world. We hope that the present workshop will be most successful, particularly in promoting future development and international collaboration in this area.

Again, our sincerest thanks to our participants for your important presence in this workshop, and we wish all of you good health and happiness.

Congratulatory Address

Mr. Nguyen Van Khang

Director, Service of Agriculture and rural Development

Tien Giang – Viet Nam

Honorable Dr. Hong-Ji Su, FFTC Representative; Dr. Sheng-Chung Huang, Director, Plant Genetic Resources Center, Taiwan Agricultural Research Institute (TARI); Dr. Nguyen Minh Chau, Director, Southern Fruit Research Institute (SOFRI); Ms. Nguyen Thi Thanh Mai, Principal Specialist, Department of Agronomy - MARD; distinguished speakers and guests. Ladies and gentlemen! Good morning!

First of all, I would like to express my special thanks to the organizers who have given me this opportunity to give my congratulatory address. It is my great pleasure to warmly welcome all of you to our Tien Giang province for the International Workshop on "*The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources*".

I understand that all we have now about 25 persons coming from 8 countries including Viet Nam. The issues of the topic and the objectives of the workshop that you are going to work for are considerably important, particularly with the developing countries like Viet Nam. In the speedy change of social and economical condition we do many mistakes leading to the disappearance of valuable genetic resource of traditional herbs, fruit species, and many other natural variations of plants.

It is estimated that Viet Nam has about 12,000 species of higher plants but only 7,000 have been identified. In a recent assessment, Viet Nam was rated as the 16th most biologically

diverse country in the world; over a thousand medicinal plants are recognized. The conservation and evaluation work in our country do not provide an adequate counter measure to this pressure on resources. And therefore, we need more support for these jobs.

It is evident that fast economic growth with a lack of tight economic and environmental controls and also some parameters such as air pollution, acid rain, water flow, soil erosion and climate change are being affecting much on the conservation and utilization of our plant genetic resources. Therefore, I do hope that the workshop will bring to all of you some new idea of research, investigations, and management for better conservation and utilization of plant genetics resource for the sake of people in long term.

Distinguished guests,

Taking this opportunity, I would like to introduce you some information of Tien Giang province. Tien Giang is one of the major agricultural provinces of the Mekong Delta Viet Nam, particularly; it has biggest areas of fruit growing areas where Southern Fruit Research Institute exists. Population of Tien Giang is 1,698,851 people; GDP is 12,772 billion VND, of which Agriculture occupies 48%. Agronomy is of importance for instance in 2005 crops brought about 6,277 billion VND with 73.8% of total agriculture value of the province. Annual crops: 3,615 billion VND; Perennial trees: 2,603 billion VND; Food plant: 2,814 billion VND and particularly fruit tree 2,480 billion VND.

I would like to express our hospitality to the international guests and other our Vietnamese friends as well. I believe Southern Fruit Research Institute will take care of you very well and once again wish you enjoy your stay in Viet Nam. Finally, on behalf of Tien Giang Agricuture sector, I wish you a very happy and reproductive new year of 2007 and have your pleasant time with impressive experience in Viet Nam.

Thank you very much for your attention.

International Training-Workshop (2007)
The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resource
1-24

Conservation, Disease-indexing and Utilization of Pathogen-free Citrus and Banana Genetic Resources in Taiwan

By
Hong-Ji Su[1], A-Shiarn Hwang[2], Su-Yin Lee[3], and Chih-Ping Chao[3]

Abstract

Virus and virus-like diseases (greening) of citrus and banana have been causing considerable damage to fruit production worldwide due to their systemic invasion and common spread through infected budwoods, vegetatively propagated seedlings, and transmission by insect vectors. The heat-tolerant form of greening locally known as Huanglungbin (HLB) in China has seriously affected citrus trees in the tropical/ subtropical regions of Asia, and recently in the North and South America. The HLB caused great damage to the citrus industry by shortening tree lifespan and lowering fruit quality. Citrus tristeza virus (CTV) is the most common and destructive virus in the western hemisphere, and has a worldwide distribution. Citrus tatter leaf capillovirus (CTLV) has a potential to become prevalent when trifoliate orange and its hybrids are used as rootstocks. The citrus exocortis viroid (CEV) occurs in many countries, and its commercial effects are primarily on trees grafted on trifoliate orange and some its hybrids, and Rangpur lime. The bunchy-top virus (BBTV) disease has been the common and destructive virus disease of banana since the beginning of this century in Asian and Pacific regions. The banana mosaic caused by CMV has become epidemic after common cultivation of tissue-culture plantlets. Banana streak badnavirus and bract mosaic potyvirus have newly spread to many banana areas in the Pacific regions in recent years. These systemic diseases have become one of the serious constraints for the industry of citrus and banana in the Asian and Pacific region.

These diseases are generally controlled by the integrated control measures including cultivation of pathogen-free seedlings, elimination of inoculum sources and prevention of secondary spread by vector insects. In view point of germplasm utilization, establishment of pathogen-free nursery system is primarily important for preventing prevalence of these diseases. National scheme of citrus pathogen-free nursery system is consist of PF citrus

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foundation in Chia-Yi Agriculture Experimental Station repository and the approved nurseries in private sector and fruit cooperative. The production and utilization of PF-citrus seedlings has been performed under citrus sapling certificate regulation. The virus-free banana cultivars of economic importance obtained through virus-indexing and tissue culture under heat-therapy temperature have been kept in the foundation screen-house for mass production of PF banana TC-plantlets in Taiwan Banana Research Institute. The PF citrus and banana seedlings are commonly cultivated for improving the agro-industry in Taiwan. The precise and rapid indexing techniques are indispensable for management of pathogen-free nursery system and health management of citrus and banana plants in the field. Development and application of molecular diagnostic probes including monoclonal antibodies, DNA probes, and primer pairs for polymerase chain reaction (PCR) were made for formulating indexing techniques including ELISA, rapid diagnostic strips, PCR and RT-PCR to be discussed in this presentation . The establishment of virus-free nursery system for controlling citrus and banana virus diseases has been properly performed through shoot-tip micro-grafting, and tissue culture via virus-free stock indexing and heat-therapy with tissue culture in Taiwan.

Introduction

Citrus and Banana are the most important fruits for local consumption and export in Taiwan and the other Asian countries. Citrus production with so many cultivars, are the most important industries for fresh fruit consumption, and juice processing world-wide particularly in the tropic and subtropical areas. Banana and plantain (*Musa* spp) are the important crops for local consumption as fresh fruit and staple food, and export in the Asian, African and American countries. Some systemic virus and virus-like diseases have been causing considerable damage to fruit yield and quality, and have became one of the serious constraint for the production of citrus and banana world wild.

Citrus greening (Huanglungbin = HLB) was first reported in 1947 from South Africa. The psyllid borne virus-like disease caused by *Liberibacter asiaticus* has been devastation citrus industry in Taiwan since 1951. This disease has been prevalent in tropical and subtropical citrus areas in Asia and South Africa, and appeared in North and South America recently. This disease caused great damage to the citrus industry by shortening tree lifespan less than 10 years. Most citrus cultivars, except pummelo, were already susceptible to the Asian form of HLB before the 1970s. However, pummelo became infected by a new GO strain in Taiwan and Southeast Asia since 1971. **Citrus tristeza closterovirus (CTV)** was assumed originated in China quite a long time ago. The CTV caused a great damage to citrus industry in South America and Africa in the 1920s and has a worldwide distribution. Some new strains of CTV have evolved in South America and Asia in recent years, and have been causing severe

stem pitting, stunting and affecting fruit yield and quality. **Citrus tatter leaf capillovirus (CTLV)** was first found in 1962 in Mayer lemon introduced to California from China. Several lines of Satsuma mandarin grown in Japan have been found to be infected by CTLV since 1975 (Miyakawa, 1980). The virus has a potential to become prevalent when trifoliate orange and its hybrids are used as rootstocks. The CTLV has been commonly in most citrus cultivars without significant symptom in Taiwan. Exocortis is a bark-scaling and tree-stunting disease caused by **citrus exocortis viroid (CEV)**. The disease was first described as scaly butt in 1948 in Australia. The casual agent occurs in many countries, and its commercial effects are primarily on trees grafted on trifoliate orange and some its hybrids, and Rangpur lime. The CEV was scarcely found in sweet orange and Eureka lemon trees rooted on Rangpur lime in Taiwan. The greening disease commonly with mix-infection of citrus tristeza and tatter leaf viruses, causes sever yellowing and decline, and ultimately death of citrus trees.

Banana bunchy-top babuvirus disease has been the common and destructive virus disease of banana since the beginning of this century in Asian and Pacific regions. Several occurrence of outbreak become the limiting factor for banana industry in Taiwan during the past decades. **Banana mosaic** caused by **cucumber mosaic cucumovirus (CMV)** was first reported in New South Wales in 1929 and in Central American in 1957. This disease had become prominent in Taiwan in 1974, and severe outbreaks occurred recently after common cultivation of tissue-culture plantlets. **Banana streak badnavirus disease (BSV)** was first described in Mysore (AAB) from Ivory Coast in 1974. The BSV was found worldwide in most of the banana-growing areas in recent years, and had potential for spreading and becoming epidemic. There is the potential for serious yield losses with some virus isolates. Plant death was reported in Africa. Disease incidence varies between countries and this may be related to strain differences and vector activity. The virus infection was recently (1994) found in exotic Mysore and Latundan, and native wild banana in Taiwan. **Banana bract mosaic disease** was first noted in 1979 in Mindanao, the Philippines. It has subsequently been widespread throughout the Philippines. The disease has also been found in India, Sri Lanka, Vietnam, and Western Samoa. A potyvirus, **banana bract mosaic virus (BBrMV)** isolated from infected plants, has been assumed to be the causal agent. Yield losses of up to 40%in Cardaba (ABB) and Lakatan (AA) have been recorded in the Philippines. Particularly, the most important cultivar, Saba (ABB) is commonly infected. The virus has been found in dwarf Cavendish (AAA) seldom in Taiwan recently.

These systemic diseases are transmitted by vegetative propagation of seedlings such as sucker, cutting, tissue culture plantlets and bud-scion for grafting as well as insect transmission. These diseases are generally controlled by the integrated control measures including cultivation of pathogen-free seedlings, elimination of inoculum sources and prevention of secondary spread by vector insects. Establishment of pathogen-free nursery

system is primarily important for preventing prevalence of these diseases (Su and Chen, 1991). Shoot-tip micro-grafting (STG) of citrus and STG combined with heat therapy have been performed for obtaining virus-free foundation stock of citrus in Taiwan (Su and Chu, 1984). The virus-free nursery system for controlling banana virus diseases has been performed by tissue culture via with virus-free stocks indexing and heat-therapy with tissue culture in Taiwan. Pathogen-free citrus and banana germplasm foundation have been established for production and cultivation of pathogen-free citrus seedling and banana TC-plantlets.

The precise and rapid indexing techniques are indispensable for management of pathogen-free nursery system. Development and application of molecular diagnostic probes including monoclonal antibodies, DNA probes and primer pairs, were made for formulating indexing techniques (Tsai and Su, 1991; Hung, Wu and Su, 1999). As a rule, a virus disease and virus-like disease are diagnosed by characteristic symptoms and bioassay with indicator plants. Nevertheless, disease indexing and detection of the causal agents are precisely and rapidly performed by enzymelinked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and reverse-transcription (RT) PCR with molecular diagnostic probes.

Disease Indexing of Citrus Greening and Virus Diseases

1. Citrus greening pathogen is commonly detected by polymerase chain reaction (PCR) analysis

Detection of HLB in symptomless citrus plants is accomplished by PCR test which has been commonly applied to indexing citrus foundation stocks and pathogen-free seedlings (Su, Hung, Wu, 1995). The sensitivity of HLB detection is uplifted by development and application of PCR analysis with adequate primer pairs (F: CAC CGA AGA TAT GGA CAA CA; R: GAG GTT CTT GTG GTT TTT CTG) (Huang, Wu and Su, 1999). The PCR-amplified DNA products are analyzed by ordinary electrophoresis (Fig. 1). (Protocol referring to the following chapter on the detection of banana viruses).

2. Citrus tristeza virus is commonly detected effectively by ELISA with polyclone and monoclonal antibodies. Development and application of monoclonal antibodies through hybridoma technology was made for differentiation certain strains of CTV (Tsai and Su, 1991). Rapid diagnostic strip has developed with monoclonal antibody for CTV detection. The globulins labeled with colloidal gold, are used for preparing the strips. The virus antibodies-secondary anti-mouse antibodies are fixed in upper part of chromatographic paper strip. (ELISA protocol referring to the following chapter on the detection of banana viruses).

Protocol of diagnostic strips:

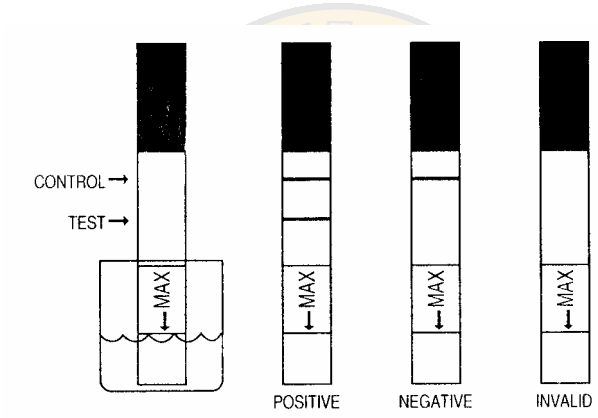
Collect 0.2~0.3 grams of tissue specimen from several leaves by cutting into pieces into an eppendorf tube.

(1) Grind the tissue pieces within 0.8ml of extraction buffer in an eppendorf tube by using bamboo/ wooden stick.

(2) Remove the test strip from its protective pouch (bring the test to room temperature before opening the pouch to avoid condensation of moisture on the membrane).

(3) Immerse a strip in the extraction buffer solution in eppendorf tube with arrow end pointing toward the solution not over labeled antibody pad. Do not immerse the strip over the MAX line.

(4) Wait for pink-colored bands to appear. Depending on the concentration of CMV, positive results may be observed within 2~3 minutes. However, to confirm negative results, the complete reaction time of 20 minutes is required.



Interpretation of results:

(1) POSITIVE: Two distinct pink-colored bands will appear, one in the plant test region and one in the control region.

(2) NEGATIVE: Only one pink-colored band appears in the control region. No apparent pink band appears in the plat test regions.

(3) INVALID: A total absence of pink-colored bands in both regions. If neither a test band nor a control band appears on the membrane, the test should be considered void. Improper testing procedures or deterioration of reagents probably occurred.

3. Citrus tatter leaf virus detection: ELISA with monoclonal antibodies has been developed, however its sensitivity of detecting the virus is low. Reverse transcription PCR (RT-PCR) was formulated by using cDNA primer pair (F: GGA AGA CTC ACA TAG ACC CG; R: TAC TCT

CCG AAC CTG CCT C) derived from the sequence of CTLV (Ohira *et. al*., 1995) for detecting the virus more rapidly. (RT-PCR protocol referring to the following chapter on the detection of BBrMV virus).

4. Citrus exocortis viroid is detected by RT-PCR with cDNA primer pair **(**F: GCT CCA CAT CCG ATC GTC; R: TGG ACG CCA GTG ATC CGC**).** (RT-PCR protocol referring to the following chapter on the detection of BBrMV virus).

Establishment of Pathogen-free Citrus Foundation Stock and Nursery System

In order to control the systemic virus and greening disease of citrus, the national scheme of virus-free nursery system was initiated, and conducted under joint program of R and D including NTU, TARI, and TBRI in Taiwan since 1983.

1. **Micro-grafting for Obtaining Pathogen-free Citrus Foundation Stocks.**

An improved method of shoot-tip micro-grafting (STG) was developed by using triangle-hole cut (Su and Chu, 1984) instead of inverted T cut described by Murashige *et al*. (1972). The STG program and health indexing were conducted in the Plant Virology Laboratory, National Taiwan University. The outline of STG schedule is as follows (Fig. 2):

Etiolated stock seedling grown on solid media
(Troyer citrange or other rootstock, 2 week-old in vitro)
↓
Micro-grafting shoot-tip into rectangular triangular hole,
Or V-cleavage of decapitated seedling
↓
Culture on liquid medium, incubate in the dark for 2 days.
↓
Growing in growth chamber or green house (28-30^{o}C)
Under cheese cloth shading for 2 weeks.
↓
Growing under uncovered condition for 2-3 weeks.
↓
Double grafting to potted rootstock seedlings
(Rough lemon, or other rootstock), and cultivate in green house
↓
The solid and liquid media were prepared following Murashige *et al.* (1972).

↓

※ Preparation of Citrus STG Medium (pH 5.7)

(1) Solid medium for growing STG seedling: MS salt mixture (Gibco BRL), 4g; Sucrose, 20g; ddH_2O, 1L; Agar, 1% (10g)

(2) Liquid medium for growing STG seedling: MS salts, 4.2g; Sucrose, 40g; growth factors (1L/100x stock: i-inositol, 100mg; thiamine-HCl, 0.2mg; pyridoxine-HCl, 1mg; nicotine acid, 1mg)

↓

2. National scheme of citrus pathogen-free nursery system

Pathogen-free citrus foundation stocks. The stocks are produced by means of improved method of shoot-tip grafting and heat-therapy in the Plant Virus Laboratory, National Taiwan University (PVL/NTU) and the Chia-Yi Agricultural Experimental Station of Taiwan Agricultural Research Institute (CAES/TARI) (Fig. 3). The STG-seedlings indexed as pathogen free by PVL/NTU, are used as foundation stock, and are transferred to the National Pathogen-free Citrus Foundation Stock Collection in screen-houses in CAES/TARI.

Scion-Propagation parent trees. Pathogen-free bud-woods are multiplied in scion-propagation trees derived from foundation stocks in insect-proof screen-house of CAES/TARI, the Taiwan Fruit Marketing Cooperative (TFMC) or approved nurserymen.

Pathogen-free citrus nurseries. Pathogen-free seedlings are produced by using pathogen-free scions derived from the parent trees in the Healthy Nursery Screen-houses operated by each branch office of the Taiwan Fruit Marketing Cooperative (TFMC) and approved nurserymen.

Citrus growers. The healthy certificated citrus seedlings are transplanted to the orchards, and protected by integrated control measures.

Note: The act of citrus bud-wood certificate has been established the production of pathogen-free citrus seedling in Taiwan since December of 2004.

3. Production of Pathogen-free Banana Seedling

These diseased might readily prevail if the mother stocks are infected latently with the causal viruses. Production of virus-free seedlings including suckers and tissue-culture (TC) plantlets through pathogen-free nursery system has to be managed by strict implementation of health indexing. The virus-free nursery system for controlling banana virus diseases has been properly performed through tissue culture via virus-free stock indexing and heat-therapy with tissue culture in Taiwan. The virus-free TC-seedlings of banana are widely grown for controlling banana virus in Taiwan.

Conservation of pathogen-free citrus germplasm

The citrus germplasm collection has been kept in Citrus gerplasm repository in Chia-Yi Agricultural Experiment Station, Agricultural Research Institute, Chia-Yi, Taiwan (Table 1).

Table 1. Citrus germplasm collection in Taiwan

Horticultural classification	Cultivars free from specific-pathogens	Cultivars not indexed
1. Mandarin		
Common mandarin	Bower, Dancy, Clementine, Encore, Esparta, Fremont, Genshokan, Guapile, Kara, Kinnow, Parson's special, Pixie, Ponkan, Early Ponkan, Sunburst, Sun Keim Woon	Drafted Ponkan
Satsuma	Okitsu No.3, Okitsu Wase, Miho Wase, Miyamoto, Nanka No.20, Emoto, Kowa, Nichinan-1	Yonezawa, Miyagawa Wase, Yamakawa, Kinho 1, Kinho 2, Kinho 3, Ohzu, Imamura, Aochima, Toman
Small fruit mandarin	Cleopatra, Depressa, Sunki,	Calamondin, Rangpur lime
2. Sweet orange		
Common	Fukuhara, Hong-Jian, Jaffa, Joppa, Liu-cheng, N. Hamlin, Suenaga, Parson Brown, Pera, Pineapple, Shamouti, Selectra, Sugar, Sun Wickom, White Siletta, Fukumatobeni	Seedless Liu-cheng, Mars early, Red Siletta, Tarocco, Trovita,
Valencia	Olinda, Cutter, Campell, Frost, Liu Gim Gong	Rhode Red
Blood	Moro, Sanguinella	
Navel	Dream, Frost, Morita, Ogawa, Fukumoto, Shirayanagi, Washington	Cara cara, Omishima, Yoshita
3. Tangor	Hai-Li, Iyo, King, Kiyomi, Murcott, Tankan, June-Tankan, Nova	Temple, Ortanique
4. Tangelo	Minneola	Orlando
5. Grapefruit	Duncan, O P Davis, Marsh, Frost Marsh, N. JBC 430 Marsh, Shamber, Star Ruby, Orablanco	Little River, Red Mexican, Thompson Pink, Red Blush
6. Pummelo	Kao Pan, MatoWentan, Thai White, Thaired pummelo,	Chandler, Mitsuyu, Red Wentan, Toyu, Wanpaiyu, Webber, Kaopan Yoshikawa Wentan,
7. Lemon	Eureka, Lisbon, Monachello,	Teresa, Villafranca
8. Lime	Bearss, Mexican	
9. Kumquat	Hong Kong, Oval, Meiwa,	Chang-shou
10. Citron	Buddda's hand, Etrog	
11. Rough lemon	Schaub	Milam, Common rough lemon

12. Sour orange	Australia, Chinotto, Common, Varigated sour, Bergamote	
13. Sweet lime	Palestine	
14. Trifoliate and hybrid	Common trifoliate, Rusk, Swingle, Troyer	Carrizo, Flying Dragon, Pomeroy
15. Miscellaneous	Excelsa, Meyer, Kawano, Shiranui, Natsudaidai, us 119	Beni-Hassaku, Excelsa, Hassaku, Hystrix, Hyuganaysu, Kimikan, Kinkoji, Konijimi, Kotokan, Kabuchi, Yuzu

*Germplasm free from greening pathogen, citrus tristeza virus and citrus tatter leaf virus.

The pathogen-free citrus cultivars obtained through shoot-tip micro-grafting and disease-indexing made in Virology Laboratory, Dept of Plant Pathology, NTU and CAES, Have been conserved in the Pathogen-free Citrus Foundation Screen-house of CAES. Pathogen-free citrus seedlings are produced by using the PF-scions supplied from the Foundation Center.

The pathogen-free nursery system has been properly performed through improved techniques of shoot-tip micro-grafting for obtaining virus/HLB-free citrus foundation stocks, and then production of healthy citrus seedlings under sapling certificate regulation in Taiwan.

Disease Indexing of Banana Virus Diseases

The reliable, sensitive and rapid indexing techniques are indispensable for management of pathogen-free nursery system and health management of banana plants in the field. Development and application of molecular diagnostic probes including monoclonal antibodies, DNA probes, and primer pairs for polymerase chain reaction (PCR) were made for formulating indexing techniques (Wu & Su, 1990; Su et al., 1997).

1. ELISA with monoclonal antibodies against BBTV and CMV.

It is difficult to purify a virus from virus-infected banana plant tissues due to high content of phenolic latex (Wu & Su, 1990; Wu et al., 1997). Therefore, it is better to produce monoclonal antibodies (McAb) of unique quality against banana viruses through hybridoma technique with impure virus preparation as immnogen. High specific monoclonal antibodies with high titer were prepared against BBTV and CMV respectively. Enzyme-linked immunosorbent assay (ELISA) with different McAbs was developed for virus detection and differentiation of the virus strains (Su *et al*., 2003). Adequate buffer solution has to be selected for virus extraction in ELISA test in order to eliminate interfering substances in banana latex

and stabilize virus extract. The adequate buffer for extracting BBTV is 0.5M Tris buffer (pH 7.5) containing 0.1% Na-DIECA, 5% sucrose and 0.5% skin milk, and for extracting CMV with 0.2M potassium-phosphate buffer (pH=7.4) containing 0.5% Na_2SO_3. Direct DAS-ELISA with alkaline phosphatase (AP)-conjugate of McAb (2H6) is used for BBTV detection, and CMV detection with AP-conjugate of McAb (7-1).

Protocol of virus detection by direct DAS-ELISA with AP-conjugate of McAb

(1) Micro-plates are coated with purified IgG of monoclonal antibody (2H6) against BBTV. Add 100ul of McAb (2ppm) in coating buffer to each well, and incubate the plates in a moist box at 37 °C for 2 hours.
(2) Wash the plates with PBS-T, 3~5 times.
(3) Add 100 µl/well of virus extracts from banana samples in virus extraction buffer at 1/5~1/20 dilution (W/V), then keep the micro-plates in a moist box at 37 °C for 2 hours or at 4 °C overnight.
(4) Wash the plates with PBS-T, 3~5 times.
(5) Add 100 µl/well of alkaline phosphatase (AP)-conjugate in PBS-T at 200x (0.5ppm) dilution. Incubate plates in a moist box at 37 °C for 2 hours.
(6) Wash the plates with PBS-T, 3~5 times.
(7) Add 100 µl/well of p-nitrophenyl phosphate (1mg/ml) in substrate buffer, and incubate plates in a moist box at 37 °C for 30~60 minutes.
(8) Read ELISA values of absorption at 405 nm.

Rapid Diagnostic Strips for BBTV and CMV

Monoclonal antibodies against banana bunchy-top virus (BBTV) and, cucumber mosaic virus (CMV) were developed by Laboratory of Plant Virology, National Taiwan University (e-mail: hjsu@ntu.edu.tw). The rapid diagnostic strips have been developed through cooperation by PVL of NUT and Meikang Biotechnology Company (e-mail: info@mei-kang.com). The globulins labeled with colloidal gold, are used for preparing the strips. The virus antibodies-secondary anti-mouse antibody are fixed in upper part of chromatographic paper strip (Protocol referring to the chapter on the detection of citrus virus).

Buffers：(Store at 4-6°C)

PBST (pH 7.4)	NaCl	8.0 g
	KH_2PO_4	0.2 g
	$Na_2HPO_4 \cdot 12H_2O$	2.9 g
	KCl	0.2 g
	NaN_3	0.2 g
	Tween 20	0.05 %
	dd H_2O	1 L
Coating buffer (pH 9.6)	Na_2CO_3	1.5 g
	$NaHCO_3$	2.93 g
	dd H_2O	1 L
Substrate buffer (pH 9.6)	Diethanol amine	97 ml
	H_2O	800 ml
	$MgCl_2$ $12H_2O$	100mg
	dd H_2O	1 L

2. Detection of banana bunchy-top virus (BBTV) and banana streak badnavirus (BSV) with polymerase chain reaction (PCR) followed by electrophoresis analysis

In order to increase sensitivity of virus detection and specificity of virus-strains differentiation, the primer pairs specific to BBTV strains were derived from the nucleotide sequences of BBTV-DNA through cloning and sequencing, and applied to formulation of the following protocol of PCR amplification followed by electrophoresis analysis. The primer pair specific to BSV quoted from J. Thomas' sequence, was applied to PCR analysis; the ordinary schedule for viral DNA/RNA extraction formulated through comparative trials, was commonly adapted for obtaining constant detection results. More recently, tissue-soak DNA extraction method mentioned below was developed for establishing rapid and inexpensive detection method of BBTV and BSV in PCR analysis.

PCR primer pairs:

※ **BBTV primer pairs:**

C1-CR:

F: 5'-GGA AGA AGC CTC TCA TCT GCT TCA GAG AGC-3'

R: 5'-CAG GCG CAC ACC TTG AGA AAC GAA AGG GAA-3'

S-CR:

F: 5'-GGG GCT TAT TAT TAC CCC CAG C-3'

R: 5'-AGC GCT TAC GTG GCG CAG CAC TAA CT-3'

SR-CR:

F: 5'-TGT CGT CGG CGA CGA AGT CG-3'

R: 5'-GGA CAT CCT CCT CCT TCA GAA GAG AGA-3'

※ **BSV primer pairs:**

F: 5'-CAA CTC AAG AGC CTA GTA TGC-3'

R: 5'-TAC CTC CGA CCG TAT TTC CAG-3'

PCR cycles for BBTV:

C-1 primer:

Step 1: 94 °C 4 min; 50 °C 1 min; 72 °C 2 min; 1 cycle.

Step 2: 94 °C 1 min; 50 °C 1 min; 72 °C 2 min; 30 cycles.

Step 3: 72 °C 10 min.

Step 4: 4 °C soaking.

S-CR, SR-CR primer:

Step 1: 94 °C 4 min; 60 °C 1 min; 72 °C 2 min; 1 cycle.

Step 2: 94 °C 1 min; 60 °C 1 min; 72 °C 2 min; 30 cycles.

Step 3: 72 °C 10 min.

Step 4: 4 °C soaking.

PCR cycles for BSV:

Step 1: 94 °C 4 min; 50 °C 1 min; 72 °C 2 min; 1 cycle.

Step 2: 94 °C 1 min; 50 °C 1 min; 72 °C 2 min; 30 cycles

Step 3: 72 °C 10 min.

Step 4: 4 °C soaking

Ordinary extraction of viral DNA (total nucleic acid):

1. Collect the leaf samples from banana plants infected with BBTV or BSV.
2. Froze the chopped tissue, 0.5g in liquid nitrogen and grind to fine powder in coffee blender or a mortar and pestle, or grind the fresh tissue directly.

3. Add 3 ml of DNA-extraction buffer* +1 % Sarkosyl (add 2.7 ml of DNA extraction buffer and 0.3 ml 10% Sarkosyl). Stir the tissue-powder suspension.
4. Transfer the suspension to a 1.5 ml eppendorf tube and incubate at 55 °C / 1hr in water bath.
5. Centrifuge the tube at 6,000 rpm / 5min.
6. Save sap supernatant (800 µl), add 100 µl of 5 M NaCl and 100 µl of CTAB / NaCl (10 % CTAB in 0.7 M NaCl), incubate at 65 °C / 10min.
7. Add equal volume of chloroform / isoamyl alcohol (24:1), mix thoroughly and spin at 11,000 rpm / 5 min (for BSV) or 6,000 rpm/ 5 min (for BBTV), save aqueous suspension.
8. Add equal volume of phenol / chloroform / isoamyl alcohol (25:24:1). Mix thoroughly and spin at 11,000 rpm / 5 min. (for BSV) or 6,000 rpm / 5 min (for BBTV), save aqueous suspension.
9. Add 0.6 volume of isopropanol to precipitate the nucleic acid. Incubate at -20 °C / 30 min. spin at 12,000 rpm / 20 min.
10. Wash pellet with 70% ethanol to remove CTAB residual. Briefly dry pellet and re-suspend in 100 µl of TE buffer. Store at -20 °C.

* DNA extraction buffer for BBTV and BSV in PCR analysis : 100 mM Tris-HCl, 100 mM EDTA, 250 mM NaCl, 100 µg / ml proteinase K (may be omitted); pH8.0 .

* DNA extraction reagents : 10% Sarkosyl (N-Lauroyl sarcosine); 5M NaCl; 10% CTAB (Hexadecryltrimethylammonium Bromide) in 0.5 M NaCl; chloroform: isoamyl alcohol (24:1); phenol: chloroform: isoamyl alcohol (25:24:1); isopropanol; 70% alcohol (ETOH); TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0).

3. Detection of banana bract mosaic potyvirus (BBrMV) by reverse-transcription PCR (RT-PCR) analysis

The formulated RT-PCR-electrophoresis analysis was applied to detection of BBrMV. The RT-PCR was developed by using the c-DNA primer pairs specific to BBrMV derived from B.C. Rodoni's sequence.

Extraction of viral RNA (total nucleic acids) from banana leaf tissues:

The present extraction procedure is almost identical with those for BBTV and BSV extraction.

cDNA primer pair for RT-PCR of BBrMV:

F: 5'-AAC GCT CAG CCT ACT TTT CG-3'

R: 5'-CAT ATC ACG CTT CAC ATC TTC A-3'

Thermocycles for RT-PCR of BBrMV:

Step 1: 50 °C 35 min; 94 °C 2 min.

Step 2: 94 °C 30 sec; 56 °C 30 sec; 68 °C 45 sec; 10 cycles.

Step 3: 94 °C 30 sec; 56 °C 30 sec; 68 °C 45 sec*; 25 cycles.

(*: 5 sec increasing ramp)

Step 4: 68 °C 7min.

Reaction mixture:

PCR reagents	**amount / tube**
ddH_2O	10µl
5x Superscript II buffer	2.5µl
10x Taq buffer	2.5µl
100 mM DTT	1.25µl
10 mM dNTPs	2µl
Taq polymerase (BRL or BerTaq)	0.2µl
Superscript II polymerase (BRL)	0.25µl
BBrMV primer pair (10 pmol /µl each)	1µl
RNA template	0.25µl

Establishment of Virus-free Banana-cultivar Foundation and Production of Pathogen-free TC-plantlets

The systemic diseases including virus diseases and Fusarial wilt might readily prevail if the mother stocks are infected latently with the causal viruses. Production of virus-free seedlings including suckers and tissue-culture (TC) plantlets through pathogen-free nursery

system has to be managed by strict implementation of health indexing. The pathogen-free (PF) banana cultivars of economic importance were obtained through virus-indexing and tissue culture under heat-therapy temperature (35 °C/3 months) (Wu and Su, 1991). The PF banana cultivars have been kept in the foundation screen-house of Taiwan Banana Research Institute (TBRI) (Fig. 4). The mass production of PF banana plantlets through tissue culture with the suckers from the foundation stocks (Fig. 6) has been properly performed in TBRI (Table 2).

Table 2. Pathogen-free foundation stocks in TBRI*

Genome type	Cultivars
AA	Kluai Khai
AAA	Taiwan Cavendish (Peichiao, Senrenchiao), Cavendish B. F (Africa), Grande Naine (Honduros), Lacatan (Jamaca), Poyo, Valery (Philippines), Vietnam Cavendish, Lacatan (Philippines), Gros Michel, William (Dwarf), Cavendish mutants resistant to Fusarial wilt (Formosana, Taichiao No. 1)
AAB	Latundan, Mas, Raja

*Cultivars free from BBTV, BSV, CMV, BBrMV, and Fusarial wilt pathogens

The virus-free banana TC-seedlings through hardening in screen-house 2 months (Fig. 7), are widely grown by farmers for controlling banana virus in Taiwan (Fig. 8).

Conservation of Banana Germplasm and Pathogen-free Banana Cultivars

The banana production was the most important agro-industry for local consumption and export during 1960~80s. Therefore, 242 cultivars of banana germplasms collection has been obtained from the domestic and foreign 34 banana-growing areas would-wild (Table 3). The cultivar collections have been kept within test tubes in germplasm bank (Fig. 5), screen-house and field conditions in Chia-Yi Agricultural Experiment Station, TARI, Chia-Yi, and TBRI, Pintung.

Banana streak badnavirus is commonly detection banana cultivars in symptom express form and virus-genome integrated form showing chloratic symptom or symptomless (Fig. 9). The cultivars introduced from INIBAP germplasm collection in Belgium, were found free from BBTV, CMV and BBrMV, but commonly infected by BSV of genome-integrated form (Table 4).

Table 3. Cultivar number of banana germplasm collection in TBRI (242 cultivars)

Genome type	Local *Musa* spp.			Foreign *Musa* spp.			Unknown origin		
	Test tube	Screen-house	Field	Test tube	Screen-house	Field	Test tube	Screen-house	Field
AAAA	0	0	0	10	10	6	0	0	0
AAAB	0	0	0	5	5	0	0	0	0
AABB	0	0	0	2	2	0	0	0	0
ABBB	0	0	0	1	1	0	0	0	0
AAA	11	11	7	87	87	70	4	4	4
AAB	1	1	0	27	27	20	16	16	16
ABB	5	5	2	14	14	9	8	8	8
BBB	0	0	0	4	4	2	0	0	0
AA	1	1	2	19	19	13	0	0	0
AB	0	0	0	2	2	2	0	0	0
BB	1	1	1	3	3	3	1	1	1
Abaca	2	2	2	4	4	4	0	0	0
Unknown	5	5	5	9	9	1	0	0	0
Total	26	26	19	187	187	130	29	29	29

Table 4. Virus detection of cultivars from Belgium (INIBAP)

	Cultivar name	Genome type	Virus detection			
			BBTV	CMV	BSV	BBrMV
I -1	Pisang Jari Buaya	AA	-	-	-	-
I -2	FHIA-01	AAAB	-	-	++	-
I -3	FHIA-02	AAAB	-	-	+	-
I -4	FHIA-03	AABB	-	-	+++	-
I -5	Williams (Bell, South Johnstone)	AAA	-	-	-	-
I -6	Cachaco	ABB	-	-	-	-
I -7	Gros-Michel	AAA	-	-	+++	-
I -8	Yangambi KM5	AAA	-	-	-	-
I -9	FHIA-17	AAAA	-	-	-	-
I -10	FHIA-23	AAAA	-	-	-	-

I -11	SH 3436-9	AAAA	-	-	-	-
I -12	TMB × 1378	ABBB	-	-	-	-
I -13	TMB × 5295-1	AAAB	-	-	+++	-
I -14	SH-3640	Hybrid	-	-	+++	-
I -15	FHIA 18	Hybrid	-	-	+++	-
I -16	FHIA 21 (#68)	AAAB	-	-	+++	-
I -17	CRBP 39		-	-	+++	-
I -18	FHIA-25	Hybrid	-	-	+	-
I -19	Pisang Ceylan	AAB	-	-	++	-

Discussion and Conclusion

(1) The production of citrus and banana fruit crops are the most important argoindustry world-wild for fresh-fruit consumption, staple food, processing and export as cash crops.

(2) Virus and virus-like diseases of citrus and banana have been causing commonly causing great damage to fruit production in fruit yield and quality due to their systemic invasion and epidemic spread through infected budwoods, vegetatively propagated seedlings, and transmission by insect vectors.

(3) In view of gremplasm utilization, establishment of pathogen-free nursery system is primarily important for preventing the prevalence of these systemic diseases.

(4) Development and application of precise and rapid indexing techniques are indispensable for management of pathogen-free nursery system and health management of PF seedling cultivation in the field.

(5) The conservation and effective utilization of citrus and banana genetic resources must be concerned to be free from the systemic virus, virus-like pathogens through disease indexing and pathogen elimination in germplasm (STG, and heat-therapy tissue culture).

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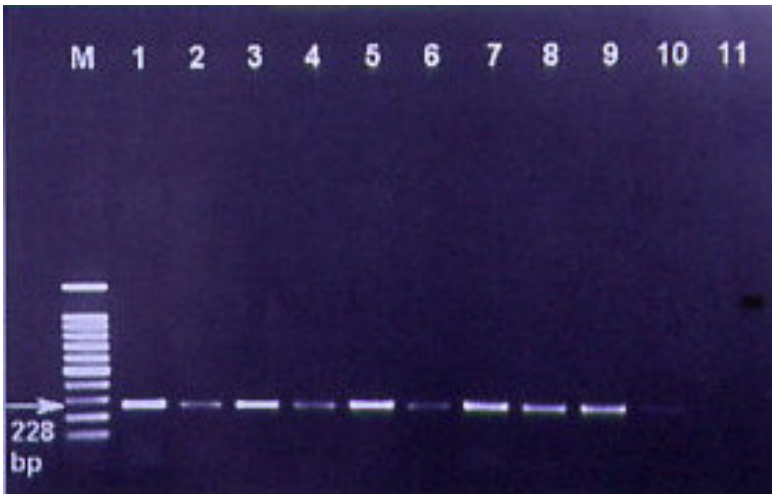
Figures



1~4: Taiwan GO isolates
5: Okinawa, Japan
6: China
7: Malaysia
8: Vietnam
9: Thailand
10: Saudi Arabia
11: Healthy CK

Fig. 1. Detection of HLB pathogen by polymerase chain reaction (PCR)/ electrophoresis analysis.

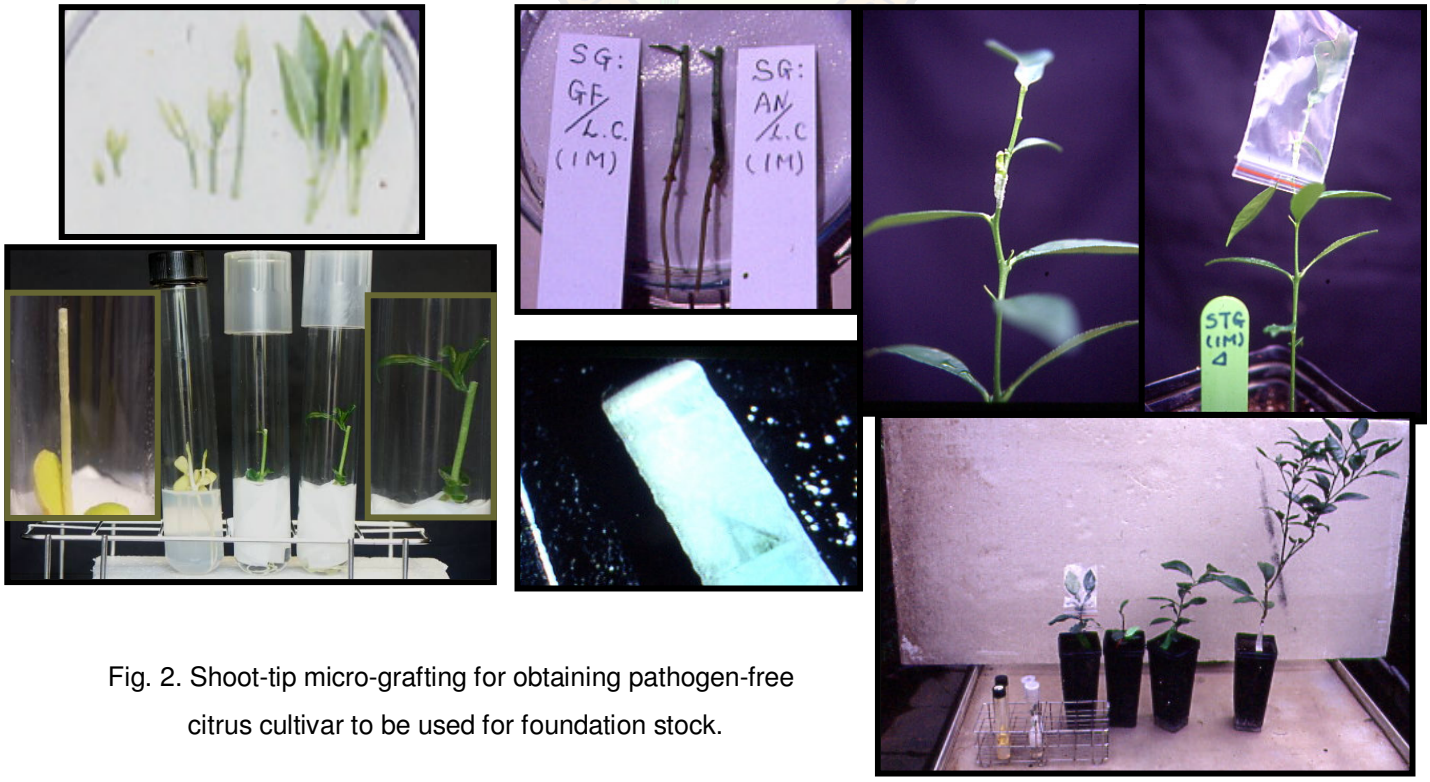


Fig. 2. Shoot-tip micro-grafting for obtaining pathogen-free citrus cultivar to be used for foundation stock.

Fig. 3. (Left) National Pathogen-free Citrus Foundation Stocks Collection Center, Chia-Yi Agricultural Experimental Station of Taiwan Agricultural Research Institute. (Right) Pathogen-free citrus foundation stocks kept in the insect-proof screen-house.

Fig. 4. Pathogen-free banana foundation stock in screen-house (TBRI)

Fig. 5. Banana germplasm collection in test tube kept at 17 °C (TBRI)

Fig. 6. (Left) Meristem culture from growing point of pathogen-free banana suckers
(Right) Multiplication of adventitious buds through tissue culture via 5~6 cuts.

Fig. 7. Mass production of PF banana TC-plantlets in tissue culture room (TBRI)

Fig. 8. Hardening of banana TC-plantlets in screen-house

Fig. 9. Banana plantation with PF TC plantlets

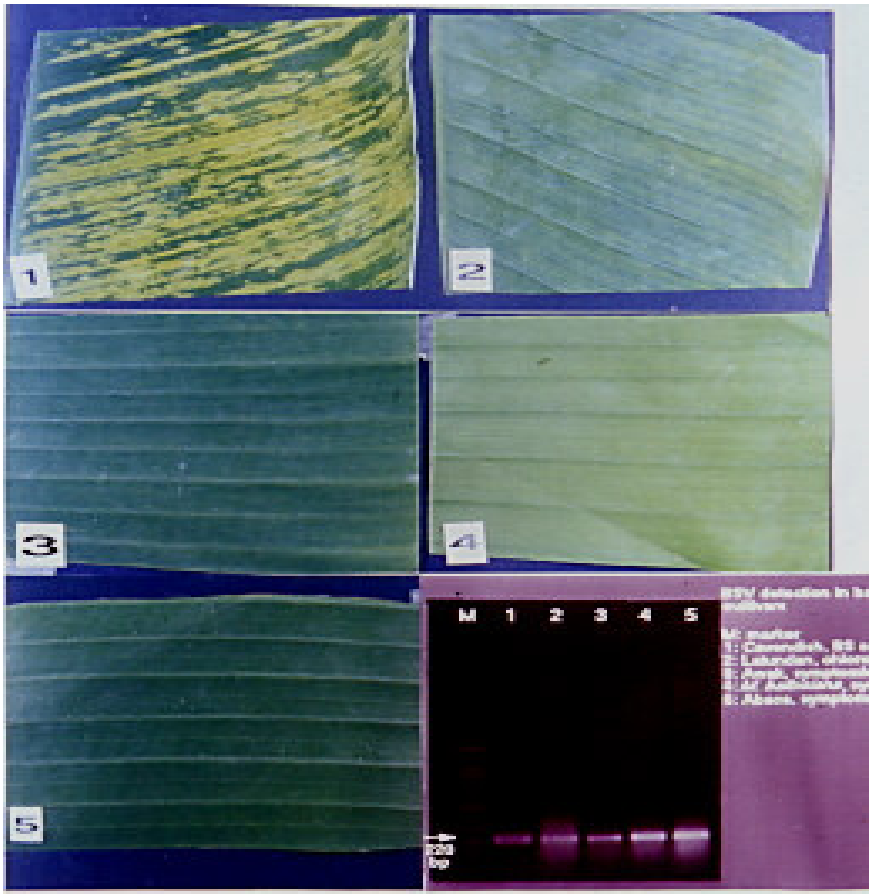


Fig. 10. BSV detection in various banana cultivars of different genome types by PCR test.

1. Cavendish (AAA)- BS severe; 2. Latundan (AAB)- chlorosis; 3. Awak (ABB)- symptomless

4. *M.balbisiana* (BB)- symptomless; 5. Abaca (*M. textilis*) - symptomless

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25-41

In-situ Conservation of Plant Genetic Resources in Vietnam: Achievements and Lessons Learned

By

Nguyen Thi Ngoc Hue[2], Luu Ngoc Trinh[1]

I. Role of *in-situ* approach in the conservation of plant genetic resources in Vietnam

Genetic resources, among which plant genetic resources share a relevant contribution, are the most important of the whole biodiversity resources. Plant genetic resources play a determinant role of ensure the sustainable agricultural development, the sustainable food production and environment protection, that is why their conservation has been calling for an increasing attention.

There are two approaches of plant genetic resources conservation, the ex-situ and the in-situ. The *ex-situ* conservation, professionally is a synonym of the seed genebank, had principally developed in developed countries and CGIAR research centers in 1960's, 1970's and 1980's. *In-situ* conservation is beneficial in that it can maintain genetic resources in natural evolution. Only in-situ conservation can truly preserve the ecological system. Promoting in-situ conservation is the primal measure towards reestablishment of the lost biodiversity.

In situ conservation is concerned with maintaining species' populations in the natural habitats where they occur, whether as uncultivated plant communities or in farmers' fields as part of existing agro-ecosystems (Brush, 1995; Bellon *et al.*, 1997). *In situ* conservation of crop plants involves the conservation on-farm of local crop landraces with the active participation of farmers. The goal of *in situ* conservation is to encourage farmers to select and maintain local crop diversity for the benefit of humankind, at the same time benefiting from it for their livelihood needs. The objective of on-farm conservation may be diverse as diverse stakeholders are involved.

In situ conservation has the potential to

(1) conserve the processes of local adaptation of crops to their environments,

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(2) conserve diversity at all levels – the ecosystem, the species, and the genetic diversity within species,
(3) improve the livelihood for farmers,
(4) maintain or increase control and access of farmers over their genetic resources,
(5) integrate farmers into the national PGR system and involve farmers directly in value addition process, and
(6) link farming community to genebank for conservation and utilization.

The importance of conservation of agro-biodiversity for future of global food security lies in its potential to supply required germplasm to crop breeders and other users. On-farm conservation will provide public and private benefits (socio-economic, ecological and genetic benefits). However, in comparison with the ex-situ, the in-situ conservation is remarkably more complicated, not only for performance, but also for conducting research activities because it closely links with agricultural development tendency, mainly the market orientation. To conduct in practical the in-situ conservation plant genetic resources demands a lot of studies on both socio-economical and biological fields of sciences

As any developing country, Vietnam needs firstly to develop the ex-situ conservation to safely conserve the important and valuable, but actually severely eroding genetic resources. Paralleling to the ex-situ, have also developed the in-situ conservation in a strategy of safeguarding the genetic resources for food and agriculture in both aspects, inter-specific and intra-specific or genetic diversity.

II. Achievements on *in-situ* conservation of plant genetic resources in Vietnam in the past period

1. Forest genetic resources and wild relatives of crop genetic resources

Vietnam government has marked the boundaries of special used forests system in nationwide. Up to now, there are 102 special used forests that were established by government under control of Ministry of Agriculture and Rural Development. Based on IUCN classification system for reserve management, Vietnam classified the special used forests system as 4 levels the following:

Level 1: National Park
Level 2: Nature Reserve
Level 3: Species/ habitat management protected area
Level 4: Protected landscape or seascape

Forest genetic resources and wild relatives of crop genetic resources have been protecting management protected areas which were set up by the Government. Forestry tree genetic resources, wild relative plants and wildlife are being preserved and exploited at 12 National Parks, 72 Nature Reserves, Species/ habitat management protected areas, 18 Protected Landscapes and Seascapes (See the Appendix 1).

2. Projects on in-situ conservation of plant genetic resources in Vietnam:

-2-1. Strengthening scientific basis of in-situ conservation of agricultural biodiversity on-farm

The project was implemented during 1999-2003. The Project has the three main objectives:

1. To support the development of a framework of knowledge on farmer decision-making processes that influence *in situ* conservation of agricultural biodiversity
2. To strengthen national institutions for the planning and implementation of conservation programs for agricultural biodiversity, and
3. To broaden the use of agricultural biodiversity and the participation in its conservation by farming communities and other groups.

The partners of Project were Hanoi Agricultural University, Tay Nguyen University and Hue University, Cantho University and PGRC-VASI. The national project coordinator (NPC) was Director of VASI, with support from Plant Genetic Resource Center (PGRC). The following activities and achievements were obtained:

Crops and Site selection

Step1: Crops such as Rice, taro and cardamon were selected for the study. The major criteria for priority crop selection were native to East Asian Center of Origin, rich intra-specific diversity and importance to farming community. Site characterizations, diversity fairs, landrace characterizations, preference ranking of landraces and baseline survey were the major activities accomplished during the project period.

Based on the results of survey, balancing all the criteria and indicators and with those agro-biological, seven macrosites were selected:

Mountain: Da bac district of Hoa binh province and Sapa of Laocai
Highland: Nho quan district of Ninh binh province and Krongno of Daklac
Lowland, Red River Delta: Nghia hung district, Nam dinh province

Lowland, Mekong River Delta: Tra cu district, Tra vinh province
Lowland central coast: Phuvang of Thuathien-Hue

Site selection for *in situ* conservation: in Vietnam based on following criteria:

Environmental (natural and human managed) diversity
High species and intra-species diversity
High cultural diversity
Sustainability of on-farm conservation
Opportunity for improvement
Accessibility

The sites differ for several characteristics and represent the ecological zones and are most appropriate for the study Example: Tracu (Mekong delta; managed by Can Tho); Nghiahung (red river delta, lowland <1.5m; high yield potential, landraces are still maintained, traditional Kinh ethnicity); Nhoquan highland (60-90m; intermediate intervention; intermediate diversity of taro and rice) and Dabac (mountainous areas; 400-700 m; no intervention; high diversity of upland rice and taro crops)

Step 2: After finishing the selection of districts, which is called as "macro-sites", it forwarded to the selection of micro-sites or selection of communes within district and villages within commune. Selection of village is the basic step of the micro-site selection. The village in Vietnam is not only an administrative unit but traditionally also a unit of culture. Cultural features such as religion, tradition, custom, occupation... vary from one to the other village, but are usually homogenous within a determined one. Selection of village is mainly based on surveyed data in company with consultation of district and commune officers and utilization of existed statistical data play a very important role.

There are following microsites were selected:

- Tat village, Tanminh commune, and Cang village, Doanket commune, these two villages belong to Dabac district, Hoabinh province;
- Yenminh village, Yenquang commune, and Quangmao village, Thachbinh commune. These two villages belong to Nhoquan district, Ninhbinh province
- Donglac vilage, Nghialac commune and Kienthanh village, Nghialoi commune belong to Nghiahung district, NamdÞnh province.
- Tra kha and Rong lon villages, Daian commune belong to Tracu district, Travinh province.
- Bankhoang commune in Sapa district, Laocai province,
- Phuda commune in Phuvang district, Thuathien - Hue province,
- Krong No district, Daclac province

Farmer household selection:

Elemental unit on "cell" of the project has been conducted. To study biological and socio-economical aspects of agro-biodiversity on-farm conservation and approaches of it implementation based on the following indicators:

- Ethnic groups of the local community
- Culture and educational level
- Number of people in a household
- Number of laborers
- Areas of land, field, pond, home garden
- Income
- Self-supply of food
- Economic status.

In each village some household were randomly taken for survey of crop resources and socioeconomic: 10 % total of households were taken from small villages; then selected from 40 to 140 farmer households.

Training and scientific exchange:

- PRA training for rapid biodiversity assessment
-Training on approaches of assessment of agro biodiversity and household economy
- Training on computer and documentation
- Training data management, 25-29 May 1998
- Vietnamese scientists had been attended some training courses and workshops on technical assistance such as data management, GIS technique.... in international institutions.

Baseline survey and conduct implementing activities:

Carried out survey 112 indicators about socioeconomic, culture and education on 600 farmer households, these aim to

- Initial PRA survey for site selection and characterization
- Initial household baseline survey to understand farmers decision making processes that influence local crop diversity
- Preferred trait assessment study for adding value to landraces
- Community biodiversity register to monitor genetic erosion and develop alternative conservation strategy.

Diversity Fairs:

Successful fairs at project sites are Dabac, Nhoquan, Nghiahung, and Tracu districts with purposes which are to:

- Recognize farmers who maintain diversity
- Locate diversity and prepare inventory of crop genetic resources (CBR)
- Understand reasons of growing diverse genetic resources in terms of use, and economic, cultural, religious, breeding and ecological values, and
- Link *in situ* conservation with *ex situ* conservation.
- Enhance conservation through exchange

Methods:

- Planning meeting with LMDG
- Date and venue
- Display of crop diversity
- Locating diversity and custodians by PRA method
- Participatory identification of farmers' cultivars
- Preference matrix ranking to understand value of the farmers variety
- Community biodiversity register-monitoring genetic erosion

On-farm diversity blocks in rice and taro crops:

Locations: Nghiahung, Nhoquan, Dabac, and Tracu sites

With purposes are to public awareness, comparison of landrace collected in the diversity fairs for agro-morphological and performance evaluation; conservation by promoting exchange of landraces.

Inventory of crop species and assessment of agro biodiversity:

Using PRA tools and questionnaire throughout following periods

1964 - 1975	At record events war time
1976 - 1987	Post war economic reinforce
1988 - 1990	First period of economic renovation
1991 to date	Economic renovation

PPB and PVS at project sites:

- Development of farmer preferred crops and cultivars diversity
- Extending the local selected varieties into farming system
- To diversify crops and crop varieties in agricultural production:

New high yield and quality varieties of target crops are given to farmers in project site as introduced genetic resources. Diversification of upland rice, lowland rice, and taro varieties are adopted by trial and extending local varieties from other areas of Vietnam.

Establishment conserved pilots at project sites:

Establish models for on-farm conservation at farmer household level for rice and taro crops in project sites; this aim to identify crop composition and to establish genetic conservation methods.

-2-2. The contribution of home gardens to *in-situ* conservation of plant genetic resources in farming systems

Home gardens hold a singular place in the culture of the Vietnamese people, and reflect their pride in their ancient tradition of gardening. The cultural value attached to home gardens has been reinforced by their important contribution to household food security in the rural, especially in the remote areas. Since 1999-2001, IPGRI's project is studying home gardens in 5 sites around the world including Vietnam. The IPGRI agreed and signed with VASI in collaborating to undertake the project "*The contribution of home gardens to in-situ conservation of plant genetic resources in farming systems*". The project located 4 sites in Vietnam.

The project has three objectives:

1. to document the species and intra-specific diversity in home gardens and the biological, cultural and socio-cultural factors that governs its distribution and supports its maintenance.
2. to development methods and approaches for including for home garden systems as components of national programs for *in situ* conservation of agro-biodiversity.
3. to support the development of "conservation through use" strategies in national plant genetic resources programs as a complement to crop based *in-situ* and ex-situ conservation.

Project's activities and achievements in Vietnam

Selection of macrosite to implement the project

Vietnam has seven agro-ecosystems. It was planned to implement the project in four of them, those are one in the north, one in central, one in eastern south and one in western south. Criteria of site selection were developed which includes:

- Diverse agro-ecosystems, in terms of species and genetic diversity as well as horizontal and vertical heterogeneity

- Cultural and socioeconomic diversity
- Importance of home gardens for livelihoods and community
- Perceived threat to traditional structure and composition of home gardens due to market forces and/or policy changes
- Existence of indigenous knowledge, skills and traditions in managing home garden system
- Community interest and cooperation
- Capacity of local research institutions
- Accessibility

The criteria used in Vietnam are designed to capture the range of socio-economic, cultural and agro-ecological variability within the country. Four sites were chosen which reflect variations in demography and ethnicity, access to markets, distance from natural ecosystems, and environmental limitations (water availability, soil fertility, etc.).

Nhoquan in Ninhbinh province was chosen as a site for northern Vietnam. The area of home garden is small and they are cultivated intensively. In Nhoquan, however, as in most places in Vietnam, the continuity of the home garden tradition was broken by war, and only rarely does one find a home garden more than 30-40 years old today.

The Nghiadan in Nghean province site is representative of the ecozone of north-central Vietnam. Its climate differs from that of the Nhoquan: Winter is the same, but summer is influenced by hot, dry winds from Laos. It is the home gardens site highest in elevation, in the midlands of Vietnam, providing both a cultural and economic contrast to the other sites.

The district of Thuanan in Binhduong province is located in the Eastern-South Basin, with a typically tropical climate with high temperatures year-round.

The district Chauthanh in Cantho province is representative of the Western-South Basin, currently called the Mekong Delta. It is a canal-based community located in the heart of the Mekong Delta.

Baseline survey and conduct implementing activities

A checklist questionnaire was carried out in November-December 1998 for up to 60 households at each site. All socioeconomic data were collected which concerned about

- Home garden size,
- Diversity,
- Household demographics,
- Age of home garden.

Key informants in the village (such as the headman) were used to select households with the most diversity and knowledge of gardening in order to provide a sample of the processes,

interaction, and uses of plants in home garden management. Eventually, 30-35 households representing home gardens in their communities were selected for a more in-depth household survey.

A baseline survey containing information on land use, home garden area, number of species grown, household ethnicity, education, yearly household income breakdown, labor and capital investment in HG per year was carried out through 1999.

A second survey entitled "*Crop species and their varieties cultivated in HG*" was completed in 2000, in which species were classified into five groups according to ecological structure and stories within the garden (Roots and Tubers, Shrubs and Climbers, etc).

An inventory of PGR was compiled from the survey with supplemental information in farmer interviews. The survey focused on uses of HG plants, sources of germplasm, commercialization and also included a distribution and transect map of the household.

A third survey focused on gathering ethno-botanical knowledge of using genetic diversity and seed flow of four key species, on farmer group discussion concerning predominant gardening aspects of top ten crop species was carried out in March-April 2001.

Base on the surveyed data, some issues were conducted:

1. Assessment and documentation of indigenous knowledge in using of local genetic diversity.
2. Study on dynamic processes of PGR evolution under HG management for establishing proper strategies of genetic diversity conservation and development.
3. Study on socio-economic aspects of gardening with emphasis on the link between HG and livelihood and gender dimension.
4. Contribution to HG diversity and adding value through PPB/PVS

Inventory crop species in Vietnamese home gardens

Followings are main results of survey and previous results of survey data analysis. A general characterization of plant genetic diversity found in home gardens of each of four selected eco-sites is presented below.

Site name	Ecological features	Avg. no of species per HG	Range of species per eco-site	Avg. garden size (m^2)	Avg. no of species/ (100 m^2)
Nhoquan	North: midland, subtropical to temperate	38,6	27-54	1407,9	2.7417
Nghiadan	North central midlands, tropical and subtropical	23,4	12-42	2771,7	0.8442
Thuanan	Eastern-South: lowland, typical tropical	50,3	36-78	2822,9	1.7819
Chauthanh	Mekong Delta: lowland, typical tropical	53,9	20-103	7500	0.7187

Research on key species.

From the 2nd year of project implementation, key species cultivated in home gardens of Vietnam had been studied. Selection of key species was based on the following six criteria:

- Widely distribution in HG throughout the country,
- Endemicness,
- Genetic diversity,
- Importance and sustainability on farmer household livelihood.
- Strong cultural connection and probability it will be maintained,
- Having a wide using way.

Four crops representative to all plants cultivated in Vietnam home gardens were selected as key species: Banana, Pomelo, Taro and Tania, and Luffa.

Gender survey and analysis

Different gender may put different values on genetic resources and their environmental function. The gender division of resources, knowledge, work and product may reflect conflict, complementary or coincidence of men and women's interest in land use systems.

Preliminary results show that men make most decision relating to cultivation of fruits and ornamentals. Fruit trees tend to generate the greatest income for the family and are highly commercialized. Ornamental plants are mostly for entertainment and need some sorts of art, which are likely to be the works of men.

In contrast, spices, medicinal, root and tuber crops seem to be the work domain of women. These crop species, in no doubt, are most important for home consumption and seem to be closely related to daily meal preparation works of women. In addition, women are responsible for the health of the whole family, therefore, they collect, plant and use of medicinal plants in home garden.

-2-3. *In situ* conservation of local rice varieties

In 1996, Agriculture and Forestry University in Central Vietnam (Hue City) started a collaborative project on *in-situ* conservation of local rice varieties with financial and technical assistance of IRRI. This is part of the Vietnam - IRRI Project "Safeguarding the preservation of the rice genepool" coordinated by the Plant Genetic Resources Center, VASI.

-2-4. Community based pilot project for the management of plant genetic resources in Vietnam

Management and conservation of biodiversity can be sustainable and effective only when the best value plant genetic resources (PGR) can be used to meet the local communities' demands, hence contributing to common benefit of all people and the state. Vietnam Agricultural Science Institute (VASI) has implemented the project "Community based on farm management of plant genetic resources" in collaboration with CIC and funding by the Italian Minister of Foreign Affairs. The project activities are being carried out in 3 different eco-sites in North Vietnam, namely the Dong Lac village, Nghia Lac Commune, Nghia Hung District of Nam Dinh province, representative for the Red River Delta; the Phu Son Commune of Nho Quan District of Ninh Binh provinces, representative for the Northern midlands, and the Chieng pan Commune, Yen Chau District of Son La Province, representative for the Northern mountainous regions of Vietnam.

The Phase 1 carried out from 1994 to 1995, surveyed the agro-biodiversity in the North of Vietnam.

In the 3 years period of Phase 2 since 2001 to 2004, the project objectives of this phase aims to restoration of biodiversity as a tool for environment protection in different agro-ecological conditions with different target species. Diversification of species existing in cropping systems of farmers' households as well as diversification of cropping ecological systems is also the concrete objectives of the project. Through creation of conditions for farmers to get access to traditional and local indigenous crops species and assisting them to know the value of these species can involve the farmers' participation in conservation and utilization of these low-cost PGR, but beneficial to local communities. Promoting community participation was main objectives of Project. Providing the stability and increasing income for the participating communities' households will serve as basis for *in-situ* conservation and increase of PGR and wild relatives of crops in farmers' communities.

Activities and achievements of the project:

- Survey of natural and climate condition at three project sites: Nghia Hung, Nho Quan, Yen Chau
- Survey the distribution and genetic diversity of the target crops at each ecosites, such as in Nghia Hung site: Traditional aromatic rice groups named “Tam thom”; In Nho Quan site: Longan, Persimmon, Mango and a local species of fruit crop named “Thanh Tra” (*Bouea oppositifolia*); In Yen Chau site with high diversity of mango species. Based on survey data, we determined existing 5 mango species in Yen Chau valley. From data, three local varieties with unique qualities were selected as the main target crops: the mango of Yen Chau District, the Kaki of Nho Quan District, and the aromatic rice of Nghia Hung District.
- In order to make three these local germplasm competitive with HYV in terms of income generated to the farmers, the following tasks were done:
    + To collect, identify, and multiply local varieties in danger of extinction in order to have it available for reintroduction in the farming system;
    + To develop low external inputs based agronomic practices for locally adapted farmers’ varieties for reducing cost of production
    + To promote local products in the internal market through campaign for raising awareness and registration of Protected Geographic Indication (PGI
- Introduction of modern propagation methods for fruit crop (especially of grafting); the cultivated protocol of aromatic rice “Tam thom” combined the traditional indigenous knowledge and modern approaches.
- Creation of an the Experimental-catalogue Field (Nursery Community) at three project sites
- Supplying Longan, Persimmon, Mango rootstocks from Nursery Community to farmers. Collecting Mango samples (including flowers, buds, leaves and fruits) for classification and for DNA extraction.

-2-5. On farm conservation of plant genetic resources at community, Can Tho University in collaboration with CEARICE

The project has been started since 1991 which conserve and develop rice genetic resources in Me Kong river delta. Objectives of the project are the following:

- To collect local rice germplasm before they disappeared by modernization policy of rice crop cultivation at Me Kong River Delta
- To take the initiative of conservation and development of rice genetic resource thanks to farmer community
- To strengthen Rice Genebank of Can Tho University in three years to be equal to the most important rice Genebank in Me Kong River Delta.

After three years, there are 1,000 samples of local rice varieties were collected. This collection was eliminated the duplicate samples remaining 812 varieties, they are being conserved in Can Tho University Genebank and evaluation as well. Beside the conservation activities, the project distributed varieties to farmer communities, 517 varieties were supplied to farmers which will be cultivated in adaptable condition and evaluated by farmers. The second phase of the project concentrated studying 6 objectives: Diversity of plant genetic resources; Conservation of PGR; Development of PGR; Seed supply system; Traditional knowledge and Policy.

-2-6. *In-situ* conservation of native landraces and their wild relative in Vietnam - VIE/01/G35

The project *In situ* Conservation of Native Landraces and their Wild Relatives in Vietnam (VIE/01/G35) which was managed by Ministry of Agriculture and Rural Development (MARD) and conducted by Institute of Agricultural Genetics (IAG), was implemented at 8 project sites in Vietnam over a period from May, 2002 to December, 2005. The project received technical advices from International Plant Genetic Resources Institute (IPGRI), was sponsored by Global Environment Fund (GEF), United Nation Development Program (UNDP), and Vietnamese Government. The aim of the project was to *in situ* preserve genetic resources of species in 6 native landraces i.e. rice, ricebean, citrus (orange, manderine, pomelo), tea, taro, and longan-lychi, and their wild relatives in 7 Northern provinces of Vietnam (Caobang, Hagiang, Tuyenquang, Langson, Hatay, Hungyen, and Haiduong). These provinces have a high level of diversity of the above native landraces and their wild relatives. They are, therefore, ideal areas for the *in situ* conservation program. To reach its target, the project has implemented a policy encouraging and creating favorable conditions for the construction of sustainable Plant Genetic Resources Importance Zones (PGR-IZs) with the involvement of communities. The project also aimed at improving education status and social perception of the community, and at propagating information relating to the expansion of genetic management zones (GMZs).

The project used community participatory methods and took every chance to obtain positive contributions of the communities to the conservation program. The specific methods are: organizing training courses, seminars, field trips, producing publications, video, pictures ect… to provide knowledge and to improve the perception of the communities on conservation. In addition, the project conducted surveys and studies on natural, economical, and social situations of the conservation zones to create scientific databases for the development of a long term conservation program. Furthermore, the project used information techniques - Geographic Information System (GIS) - to build PGR-IZs and supervise the diversity of the concerned species.

Four active components of the project: (i). Setting up Plant Genetic Resources Importance Zones through the establishment of suitable and encourageous environment; (ii) Creating favorable conditions for the operation of PGRIZs through removing obstacles and training to improve the capacity of human resources; (iii) Conducting specialized researches, analyzing and managing information to support the establishment and operation of PGR-IZs; and (iv) Improving perception capability of communities through education and propagating information to support the expansion of PGR-IZs. After 3-year operation, the project has achieved the followings:

-Ten PGR-IZs has been established. Native landraces belonging to the 6 target plant species and their wild relatives has initially been safely conserved at dynamic agro-forestry zones.

-The diversity of plant species of priority found existing in each PGR-IZ has been documented.

-Awareness in local communities on conservation has been improved and;

-Efforts of local communities in conservation and effective utilization of biodiversity has been enhanced. Eleven community participatory models of conservation which are expandable to other regions have been established.

The National Coordinating Committee of the project, in conjunction with Institute of Agricultural Genetics published a book titled: "*In situ* Conservation and Sustainable Use of Native Landraces and their Wild Relatives in Vietnam" with the aim to provide researchers in research institutions with information relating to *in situ* conservation approaches of agricultural biodiversity for sustainable agricultural production and national food security.

III. Lessons Learnt

1. What basic information needed to understand *in situ* conservation?

Following four main research questions will be providing a scientific basis for designing and planning effective *in situ* (on-farm) conservation:

1. what is the extent and distribution of the genetic diversity maintained by farmers over space and over time,
2. what are the processes used to maintain the genetic diversity on-farm,
3. who maintains genetic diversity within farming communities (men, women, young, old, rich, poor, certain ethnic groups), and
4. What factors (market, non-market, social, environmental) influence farmer decisions on maintaining traditional varieties.

Answering these four research questions together with strengthening formal and informal capacity and links has enable national programme to support farmers in the conservation and use of crop genetic resources through enhancing the social, economic, ecological and genetic benefits from local crop resources to farmers and other stakeholders.

2. Promoting in-situ genetic resources conservation together with agro-forestry ecological system conservation

Promote research on evaluation and exploitation of germplasm.

Conservation through use can only be feasible when genetic resources are in mass use. Therefore, Vietnam needs to invest on scientific research on germplasm evaluation and exploitation. The main content of this is to evaluate genetic diversity to identify and to detect valuable gene sources. Evaluation of genetic diversity needs the implementing research from molecular and agro-morphological levels to ecological adaptation. From the evaluation, good plant varieties and animal races can be selected to extend in production.

Diversity gene pool in agro-forestry production.

This is the most important measure to realize conservation through use of genetic resources, particularly for crop species. Diversifying crop gene pool in production includes diversifying crops, diversifying varieties of each crop and diversifying genes in each new breeding variety. This work is to promote sustainability of production and efficiency in agricultural environmental protection. Diversifying gene pool in production needs to be made through detailed agro-promotion missions in the national agriculture extension policy.

Major steps of in-situ conservation for each target germplasm:

1. Survey and study to identify germplasm need to be conserved, study natural, socioeconomic conditions at selected eco-zones.
2. Boundary and establishment of pilots for in-situ conservation of target crops, races, species
3. Observation of changes, evaluation of effectiveness
4. Establishment of germplasm management information system aim to exchange and monitoring
5. Increasing the indicators of richness and evenness by germplasm diversity, combination of conservation and utilization, and effective introduction of traditional germplasm.

3. Summing up orientation a strategy on in-situ conservation of plant genetic resources can be classified to the following three groups:

1. Forest plant species, wild relative plants and wildlife should be conserved at special used forests such as national parks and nature reserves.
2. Wild relative plants not in existence in the special used forests. Up to certain case, of in-situ conservation spot is set up for each species or group of species.
3. Perennial crop species, mainly fruit and cash crops, establish conservation areas in different ecological areas throughout the country which located in Vinh Phuc (Phu Ho) and Baria Vung Tau (Tan Thanh) provinces for fruit crops; Dak Lak (Buon Ma Thuat) and Nghe An (Phu Quy) provinces for cash crops.
4. Annual food crops include calorie crops and others food crops use on-farm conservation.

IV. Conclusion

Plant genetic resources play a determinant role of ensure the sustainable agricultural development, the sustainable food production and environment protection, that is why their conservation has been calling for an increasing attention.

There are two approaches of plant genetic resources conservation, the ex-situ and the in-situ. In-situ conservation is beneficial in that it can maintain genetic resources in natural

evolution. Only *in-situ* conservation can truly preserve the ecological system. Promoting *in-situ* conservation is the primal measure towards reestablishment of the lost biodiversity.

The goal of *in situ* conservation is to encourage farmers to select and maintain local crop diversity for the benefit of humankind, at the same time benefiting from it for their livelihood needs.

Diversifying gene pool in production needs to be made through detailed agro-promotion missions in the national agriculture extension policy. Exchange the germplasm is necessary to enrich the gene pool.

Major challenges are (i) to integrate the conservation of plant genetic resources with agricultural development, and in particular to conserve as much diversity as possible and the processes that give birth to it; (ii) to develop the framework of knowledge to determine where, when and how *in situ* conservation will be effective and develop a broad guideline for research and practice *in situ* conservation for national programmes that address the needs of farming community as well.

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Genetic Resources of Rutaceous Plants against the Psyllid Vector Preference and Pathogen Transmission of Huanglongbing

By

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Introduction

Huanglonbing is the most important disease on citrus in Indonesia. The disease is caused by *candidatus* Liberibacter asiaticus and spread by the psyllid vector *Diaphorina citri* and by vegetative propagation. Almost all commercial citrus are susceptible to the disease, however, some citrus relative plants were reported to be the host of the psyllid but not the host of the pathogen or none of them as the host plants of the psyllid either the pathogen. Those plants reported as non host of the pathogen but may be as or may be not as the host plants of the psyllid are promising to be used as resistant genetic resources against the disease. This report is discussing the research on citrus relative trials for the preference of *D. citri* and the transmission of Liberibacter. Furthermore, the species of *Muraya paniculata* and *M. exotica* both as the host plants of the psyllid, however, only one of them was reported as the host plant of Liberibacter. Unfortunately, the characteristics of *M. paniculata* and *M. exotica* have been in doubtfully mix up, therefore the name of *M. paniculata* in a certain report is given for *M. exotica.* Therefore this report is also discussing the characteristic of *M. paniculata* compared to *M. exotica* morphologically and molecularly.

Materials and Methods

A. Green House Experiment of Citrus Relative Trial for the Preference of *D. citri*

Disease free of *D. citri* was obtained from cultures which were maintained on *Muraya paniculata* in the control temperature glass house (CT). CT temperature for rearing was set on 28°C and relative humidity set on 60%.

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Experiment was set up in choice mode with seven species of citrus relative plants and five replications. *Swinglea glutinosa, Aegle marmelos, Murraya paniculata*, *Murraya exotica, Limonia acidisima, Triphasia aurantifolia* and *Glycosmis pentaphylla* plants were used.

Plants were placed in the transparent plastic cage (63 cm diameter, 90 cm high), covered by nylon mesh on the top. Plants arrange randomly in circular style. 20 *D. citri* adults were released in the middle of the arena in each cage. The number of *D. citri* sit on each plant was recorded twice a day for seven days, at 10 am and 4 pm. *D. citri* found to be sitting on the plants were considered as feeding on the plants.

B. Filed Trial Experiment of Citrus Relative Plants for the Preference of *D. citri* and HLB Transmission

The number of 20 different plant species (or cultivars) of Rutaceae (Table 1) were planted on the land randomly with 16 replicate blocks. The distance between plants are 2 m and *C. reticulata* cv. Siem was planted as border plants between blocks. The experiment is conducted at low land of about 100 m also in the endemic area of HLB surrounded by several citrus orchards belongs to the local farmers. The cultivation of the plants was as a standard Siem mandarin cultivation by 2 applications of fertilizer a year of 5 kg organic fertilizer/plant and 50 g or ZA/plant in the first year.

Table 1. Name of live collection species of *Murraya* spp for morphological and molecular (DNA isolation) characterization.

No.	Name of species	Origin	Number of sample
1	*M. crenulata*	Bogor Botanical Garden	1
2	*M. crenulata*	Purwodadi Botanical Garden	1
3	*M. exotica*	Bogor and around	6
4	*M. exotica*	Yogyakarta and around	14
5	*M. exotica*	Purwodadi and around	5
6	*M. koenigii*	Bogor and around	8
7	*M. koenigii*	Yogyakarta and around	12
8	*M. koenigii*	Purwodadi and around	10
9	*M. paniculata*	Bogor and around	10
10	*M. paniculata*	Yogyakarta and around	10
11	*M. paniculata*	Purwodadi and around	5

Each plant in every block was observed for the psyllid population every fortnightly and one year observation has been conducted. The HLB like symptom was observed however PCR confirmation was conducted one year after planting.

C. Characterization of *Muraya* spp.

To support the experiment on citrus relative trial for the preference of *D. citri* and the transmission of HLB characterization of *Muraya* spp. was conducted. Morphological and molecular characterizations were conducted on several samples originated from different localities in Java.

-C.1. Morphological characterization

In morphological study, parameters observed are characters such as:

a. shape (low scrub; high scrub, low tree; high plant)
b. stem (ramification; stem surface; hair at stem; stem color)
c. leaves (type of multiple leaves; maximum length of multiple leaves; maximum amount of leaflet; pair of leaflet; surface of petiole; shape of leaflet; sessile leaflet; clear hole of oil gland node; amount of oil gland per 1x1 cm; hair in oil gland; amount of hair in oil gland; hair in leaf vein; leaf edge; leaf end; leaf base; shape of leaf base; ventral; dorsal; midrib; hair in midrib; leaf vein; lateral bone; leaf thickness; petiole; hair in vein)
d. flower (type of inflorescent, amount of flower; inflorescent position; firstly blossom; pistil growth, flower size; sepal size; position of sepal; petal; shape of petal; size of petal; position of petal; size of outer filament; size of inner filament)
e. fruit (size of fruit; color of ripe fruit; fruit peel; shape of fruit)
f. seed (shape; color; color of aril; position of cotyledon at growing)
g. leaf anatomy (amount of palisade parenchyma; dense of palisade parenchyma)
h. flower anatomy (position of placenta)

-C.2. Molecular characterization

DNA was isolated from plants obtained from living collections located at Bogor (06 36' S, 106 48' E), Purwodadi (07 07' S, 110 55' E), and Yogyakarta (07 49' S, 110 22' E) using the CTAB method of Komar (1999). DNA amplification was based on the method of Karsinah (2002). The amplification was conducted in 15 L of solution consisting of 7.5 L of PCR master mix from Microzone Ltd, 1.5 L of primer 15 pmol, 4.5 L of MilliQ water, and 1.5 L of template DNA using My Cyber thermocycler (BioRad). The primers were used for amplification were OPU-3, 6, and 7, and OPN-16 and OPW-19. An initial denaturation was carried out at 95°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 30 s,

annealing at 36℃ for 30 s, and extending at 72℃ for 80s. A last cycle with extension at 72℃ for 10 min was then performed. Electrophoresis was performed at 100 V for 25~30 min in a 1.0 % agarose gel in which ethidium bromide had been added. The amplified DNA fragment patterns were visualized using a UV transilluminator and photographs taken.

D. HLB infection on Muraya spp.

Natural infection of HLB on Muraya was observed elsewhere and confirmed using PCR analysis. Inoculation of Muraya seedlings using *D. citri* was conducted in the greenhouse. Seedlings at the age of 5~6 weeks were invested with infectious D. citri obtained from endemic area of citrus HLB then observed for the symptom development and PCR confirmation.

Results

A. Green House experiment of citrus relative trial for the preference of *D. citri.*

The number of *D. citri* adult stay on citrus relatives is showed in Figure 1. *D. citri* feed on *Aegle marmelos* was constantly highest than others. On *Glycosmis pentaphylla* the number of D. *citri* tend to be decreased, but on *Limonia acidisima* tend to be increased.

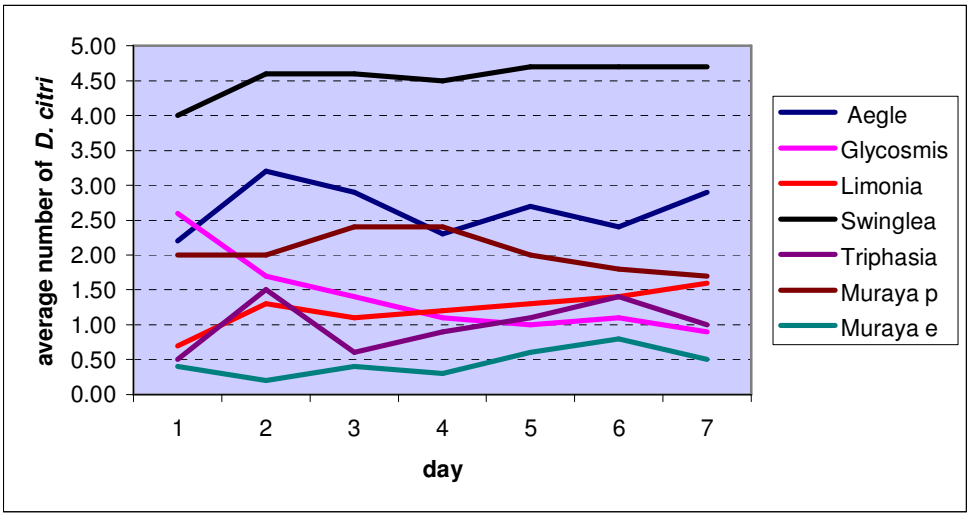

Figure 1. The fluctuation number of *D. citri* adult stay on citrus relatives which is considered as a feeding site

D. citri showed significantly ($P < 0.001$) highest preference to feed on *Swinglea glutinosa*. The least interest for feeding was on the *Murraya exotica* and no plants were found to be rejected by *D. citri* for feeding, as showed in Figure 2.

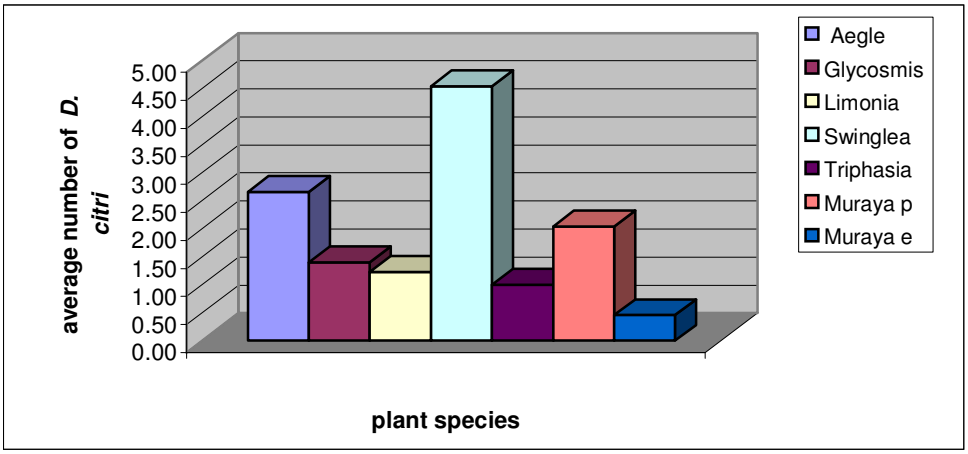

Figure 2. The average number of *D. citri* feeding on citrus relatives

B. Filed trial experiment of Citrus relative plants for the preference of *D. citri* and HLB transmission

From 20 species/cv of Rutaceae, the species/cv which were not visited by *D. citri* in year 2006 included *Aegle marmelos, Limonia acidisima*, *Feroniela lucida*, *Glycosmis pentaphylla*, *Clausena harmandiana,C. lancium,C. reticulata* var Grabag, and *C. r.* var. Siem. Those two varieties of *C. reticulata* were probably escaped from *D. citri* visit due to the same or very similar to those border plants which are *C. r.* var. Siem. There were 12 species/cv which were visited by *D. citri* in the field trial of citrus relative plants tested. The data on the population of *D. citri* on those species/cv is showed in Figure 3. The *D. citri* population on those species/cv of citrus relatives were not related to the transmission of HLB as PCR confirmation was conducted with no positive results was found. Further observation is being continued for the following years both for the population dynamic of *D. citri* and HLB transmission.

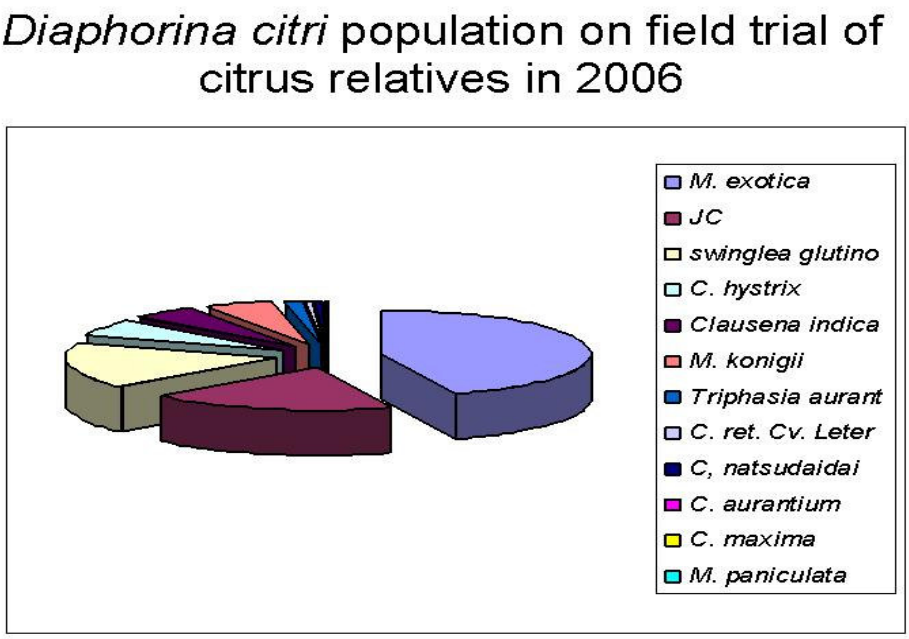

Figure 3. *Diaphorina citri* populations on field trial of citrus relative in 2006

C. Morphological Characterization of *Muraya* spp.

-C1. Characterizations

M. koenigii is separated from three other species of *Murraya* because the species has spread leaf sessility. Leaf sessility is fixed character because it is not influenced by outside factor. *M. crenulata* is distinguished to *M. exotica* and *M. paniculata* based on hair structure present in surfaces of stem, branch, leaf and petiole as well as in oil gland. Existence of hair indicates genetic nature, because the hairs have functioned as protector, reducing evaporation. It is also for even panicle inflorescent shape and its position only in stem end. Character that is also used to distinguish *M. crenulata* agrees with character used in Hartley (1993)'s description.

Separation of *M. exotica* and *M. paniculata* is due to leaf shape, flower growth, fruit shape and seed shape. The characters are nature based on genetic nature. Use of the characters supports Stone (1985) and Uji (1994), although Backer and Bakhuizen vd Brink (1965) and Swingle (1967) stated that *M. exotica* is synonym of *M. paniculata*, and Huang stated it as variety (*M. paniculata* var *exotica)*. Therefore, it is clear that in morphological manner *M. exotica* and *M. paniculata* can be distinguished as different species. To get more certainty, quantitative analysis using numeric taxonomical approach was done.

-C2. Molecular Characteristics

RAPD analysis using primers OPM-16 and OPW-19 produced DNA banding patterns that support the separation of *M. exotica* and *M. paniculata* into two species. However, as the origins of the material in Indonesia are unknown, the possibility exists that it may have come from one original introduction. Therefore, further RAPD analysis was conducted using primers OPU-3, OPU-6 and OPU-7. Amplification with these primers gave variable results and there was no consistency in the banding patterns produced from DNA extracted from different accessions of the two species. Therefore, the accessions of the two species all appear to have come from different sources and supports the separation of the two species based on data using OPM-16 and OPW-19.

D. HLB infection on *Muraya* spp.

HLB infection naturally was found on *M. exotica* in Denpasar Bali and PCR confirmation was positively observed. Inoculation on *Muraya exotica* was resulted on the symptom development and positive PCR confirmation but not on *M. paniculata*.

Discussion

Citrus greening is very severe disease and infecting mostly all commercial citrus in Indonesia and other countries. Genetic resources for resistance against the disease are needed. Therefore the experiments of citrus trial for the preference of HLB psyllid vector *D. citri* and the transmission of HLB are needed to be further conducted. The experimental results discussed in this paper are that from 20 species/cv of Aurantioidea plants, the psyllid in the field has preference on certain species or citrus cultivars (Figure 3). However, the species which were not visited by the psyllid in the field experiments were visited in the green house experiment using cages. Therefore, it was understood that psyllid landing on a certain species may be having several purposes which were only temporary visit, for feeding and laying eggs or maybe for protection of avoiding from hazardous environment. The most important reason for the psyllid visiting the species is when they feed on them because it will be depositing the HLB pathogen inoculums. The successful of transmission naturally and artificially by psyllid was on *M. exotica*, and in the field. We found the highest population was found on that species. Furthermore, the report from Brazil HLB suggested that several times infected Muraya were found, which they claim as *M. paniculata* but on our rough description we know that the plant was *M. exotica* (Silvio Lopes, personnel communication). Further collaboration to identify the species of Muraya naturally infected by HLB in Brazile and the genetic diversity of the pathogen is progress.

Further research may lead to the psyllid behavior visiting citrus relative plants related to feeding purposes and the durability of infection of HLB in the citrus relative plants. The resistance gene may obtain from the plants which the psyllid feed on and transmission the pathogen; however the disease is inhibited to develop further.

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The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources
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Current Status of Conservation and Utilization of Tropical Plant Genetic Resources for Food and Agriculture in Malaysia

By

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Introduction

Malaysia is regarded as one of twelve "mega-biodiversity" countries in the world. Its tropical rainforest has been endowed with exceedingly rich flora and fauna biological resources. The hot and humid tropical climate with the generally ample sunshine and the good availability of rains throughout the year is the key factor in influencing the survival of these biological resources over times. It has been estimated that not less than 15,000 species of vascular plants, 290 species of mammals, 150,000 species of invertebrates and an enormous number of microbial populations exists in the forests (Anon, 1997).

General Issues on Plant Genetic Resources

Local biologists agree that many of the plant genetic resources (PGR), particularly the rare and wild species of food crops have not been fully studied and very little is known on their actual distribution and potential use. Some species are near to extinction even before they are documented. Another similar situation also exists with the disappearance of many land races, traditional varieties, natural mutants and specific ecotypes. The process of urbanization, agricultural development and logging activities are inevitable factors that led to such a gradual loss of crop biodiversity.

Systematic effort to inventorize and document the range of crop biodiversity including

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old land races, traditional varieties, natural mutants and specific ecotypes is also lacking. There is also the question of securing and keeping the materials in safe condition in field or seeds genebank. Similarly the issues of inadequate characterization, evaluation and utilization of biological genetic resources have often been raised. It should be realized that only by taking advantage of the genetic diversity can scientists develop improved and adaptable varieties. There is a need for urgent action to mobilize efforts in mitigating such loss of genetic diversity and overcoming various issues through their judicious management and sustainable utilization of biological resources.

Agricultural Research and Research on PGR

Agricultural research in Malaysia is carried out by a number of public and private sector organizations. Each of these has differing scope of work and functions. Of particular importance the relevant research agencies include Malaysian Agricultural Research and Development Institute (MARDI) that conduct research on all crops (except oil palm, rubber, cocoa and forest trees) and livestock, Palm Oil Research Institute of Malaysia (PORIM) on oil palm, Malaysian Rubber Development Board (MRDB) on rubber, Malaysian Cocoa Board (MCB) on cocoa and Forests Research Institute of Malaysia (FRIM) on timber and forest trees. Besides, both Department of Agriculture in the state of Sabah and Sarawak have their own research division with specific focus to the needs of agriculture in their respective states. Similarly research activities on specific areas of crops and agriculture do also involve many local universities and non-governmental organization.

Being the lead agency in spearheading the agricultural technology development in the country, MARDI has been identified as the national focal point for research and management of agro-biodiversity since the year 1996. Since then, Management and Utilization of Biological Resource Programme under the Strategic Resource Research Centre of MARDI have been given the task to undertake the national responsibility in the conservation, characterization, evaluation and utilization of crop genetic resources. The main objective within the programme is to mitigate the loss of agro-biological resources in the country through their judicious management and utilization in support of sustainable national food and agriculture development. In addition, the other functions of the programme are to gather and disseminate information on the nation's agro-biodiversity; to conduct research and development in areas relevant to management and sustainable utilization of agro-biological resources and to position MARDI as the national reference center for agro-biodiversity.

Recognizing the importance of plant genetic resources for food and agriculture, MARDI have been actively engaged in the exploration, collection, conservation and utilization of crop genetic resources, thus contributing to the activities of Convention of Biological

Diversity (CBD), Global Plan of Action (GPA) for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (PGRFA) as well as the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

Other agencies listed above also undertake research activities pertaining to the management and utilizations of PGR but confined to their respective mandated crops and scope of functions. The three crops namely oil palm, rubber and cocoa which contribute to the major agricultural export earnings from Malaysia represent introduced crop species. All PGR for these crops are basically introduced from outside the country. Thus it would not be that relevant to discuss it in this paper. In the context of CBD, ITPGRFA and GPA, activities carried out by MARDI as well as other agencies especially Dept. of Agriculture in Sabah and Sarawak affecting the conservation and utilizations of the indigenous crop biodiversity would be very appropriate and these would be elaborated in this paper.

In-situ Conservation

In-situ conservation appears to be a better option for the conservation of PGRFA. In the context of this paper "*in-situ* conservation" was taken to mean "*the conservation of ecosystem and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties*" (Anon, 1997). Implementing *in-situ* conservation plans on PRGFA would thus involve not only maintaining indigenous and wild species in their original habitat but may also include conservation of domesticated and semi-wild species at its eco-adapted sites where man's influence over many generations may have allowed it to evolve into its peculiar characteristics. In the latter, conservation could be pursued on the farmers land itself thus rendering it as an on-farm conservation but at the same time as an *ex-situ* approach as the species may have moved out of the original habitat (Wong, 2005).

- In-situ conservation of wild species

In-situ conservation of PGRFA involving wild relatives of crops in Malaysia is effectively carried out in the protected areas. However this requires large areas of undisturbed forests having, ideally, maximum species diversity and high intra-specific diversity for different species. Also, selecting large land mass as candidate genetic reserves is not easy due to a general lack of basic floristic data in the targeted areas. An overview of these legally protected areas has been given by Soepadmo (1995). The total protected areas was 5.8% (763,300 ha), 8.9% (658,824 ha) and 2.3% (288,806 ha) of the total land area respectively in Peninsular Malaysia, Sabah and Sarawak (Table 1). With respect to Sarawak, Soepadmo

also noted then that the state authorities have strong intention of expanding the protected areas to 1.14 million hectares representing about 8% of the total land area in the state.

The total protected areas of the forest habitats comprise of the gazetted National and State Parks, Virgin jungle reserves, Wildlife reserves and Bird sanctuaries as well as Protection forest (in the case of Sabah). Among the more important and larger areas where probably an extensive flora and fauna species diversity occurred include Taman Negara (434,000 ha), Endau-Rompin Park and Wildlife Reserves (90,000 ha) and Krau Wildlife Reserve (63,000 ha) in Peninsular Malaysia, Gunung Mulu Park (53,000 ha), Lanjak Entimau Wildlife Sanctuary (169,000 ha) and Batang Ai Park (24,000 ha) in Sarawak and Kinabalu Park (73,000 ha), Crocker Range Park (140,000 ha) and Tabin Wildlife Reserve (141,200 ha) in Sabah. Virgin Jungle Reserves (VJRs) are established to serve as permanent nature reserves and natural arboreta. In Peninsular Malaysia VJRs cover 19,000ha, representing various forest types while in Sabah there are over 88,000 ha.

Table1. Existing protected forest habitats in Malaysia

Type of habitat	Area (hectare)			
	Peninsular Malaysia	Sabah	Sarawak	Total
National/ State Parks	434,300	245,172	113,955	793,427
Virgin jungle reserves	19,000	88,304	-	107,304
Wildlife reserves/bird sanctuaries	310,000	143,682	174,851	628,543
Other protection forests/ conservation areas	-	181,666	-	181,666
Total protected areas	763,300	658,824	288,806	1,710,930
Total land area	13,160,000	7,400,000	12,300,000	32,860,000
Total remaining forests	6,150,000	4,520,000	8,700,000	19,370,000
Total permanent forest estate	4,750,000	3,350,000	6,000,000	14,100,000

Source: Soepadmo (1995)

Inventorying work in some of these protected areas has been initiated. For example inventories of wild fruit species, which as a group has recently attracted many interests among local biologists, have been conducted at Taman Negara, Pasoh Forest Reserve and Krau Wildlife Reserve. It was found on the average five individuals of wild fruit trees per ha as

having a 10 cm or more stem diameter and it was also noted that the species remain understorey trees that would not exceed 30 m in height and greater than 30 cm in diameter (Jong *et al*., 1973; Yap, 1980, 1982; Soepadmo, 1979; Saw *et al*., 1991). Extensive studies at Pasoh Forest Reserve further indicated that the densities of adult wild fruit trees also vary greatly between species (Saw *et al.,* 1991). For example within the genus *Mangifera* which is represented by 12 species, the adult density is 0.1 trees per ha as compared to 180 trees for *Xerospermum noronhianum.*

An inventory made on 50 ha of primary lowland rain forest in Peninsular Malaysia at Pasoh Forest Reserves (Negeri Sembilan) further showed that there were 76 fruit species known to bear edible fruits. The most diverse species found include those of the related wild species of mango (*Mangifera* , 12 species), of mangosteen (*Garcinia,* 13 spp), of jackfruit and breadfruit (*Artocarpus,* 10 spp) and of rambutan (*Nephelium*, 5 spp). Another survey inventorying the composition, distribution and abundance of wild fruit species in a 0.99 ha transect in the peat swamp, lowland and ridge forest at Sungai Dusun Wildlife Reserve, Selangor showed the area contained 16 families, 28 genera and 42 species with 359 individuals of wild fruit trees. Here the highest number of individuals belonged to the family Burseraceae. In another instance 32 edible fruit species were recorded in Belum Forest Reserve in Hulu Perak, Perak. These wild fruit tree species recorded in the jungle reserves will therefore contribute to the *in-situ* conservation.

- On-farm conservation of cultivated crops

To-date no major efforts on *in-situ* conservation for cultivated crops (which is synonymous to on-farm conservation as has been explained earlier) have been carried out in Malaysia. In fact, it represents a new approach in conservation (at least to the Malaysian researchers) as it has only been recently advocated to local scientists.

Quite a recent project that deals with the subject entitled “On-farm Conservation and Utilization of Traditional Fruit Species” was initiated and coordinated by International Tropical Fruits Network (TFNet) in collaboration with the Departments of Agriculture Peninsular Malaysia, Sabah and Sarawak, MARDI, University Kebangsaan Malaysia (UKM), International Plant Genetic Resources Institute (IPGRI) and Department of Forestry. Partial funding for the project was received from Fulbright/Humphrey Programme (Salma, 2003). The specific objectives of the study include:

1. To gather information on households involved in the cultivation of the underutilized fruits.
2. To determine the types, status and distribution of the underutilized indigenous fruits and their ethno-botanical information

3. To assess the economic value of the underutilized indigenous fruits
4. To provide information that can assist in strategic planning and project formulation for on-farm conservation of these fruits.

During the study three sites, one each in Peninsular Malaysia, Sabah and Sarawak with the most diverse fruit species were selected for further assessment on the use of the species and their respective contribution to the community livelihoods. The author noted that between 21 to 52 species of traditional fruit trees are cultivated in the selected sites. Each site was found to have differing species ranking in terms of its importance in enhancing the livelihood of the community. For example at Kampong Linkungan, Hulu Cheka in Jerantut, Pahang the three species with the highest ranking are *Garcinia atroviridis*, *Mangifera odorata* and *Parkia speciosa* while it was *Mangifera pnjang*, *Nephelium ramboutan-ake* and *Parkia speciosa* for Kampong Pintas in Beaufort, Sabah. For Rumah Kallang Changkul in Bandar Sri Aman, Sarawak the high value fruit species are *Dialium sp*., *Dimocarpus longan* var. *malesianus*, *Canarium odontophyllum*, *Parkia speciosa* and *Xanthophyllum amoenum. Parkia speciosa* was the only common species ranked as important in all the three sites. This legume is used mainly as 'ulam' (just like vegetable salad) and consumed with rice.

A recent proposal for implementation of on-farm conservation of crops has been prepared. The project is entitled "Conservation and Sustainable Use of Cultivated and Wild Tropical Fruit Diversity: Promoting Sustainable Livelihoods, Food Security and Ecosystem Services" to be supported by UNEP/GEF funding (Salma, 2005). It is targeted towards the conservation of various fruits species within the three genera namely *Garcinia*, *Mangifera* and *Nephelium* and it will be implemented in selected sites within Peninsular Malaysia, Sabah and Sarawak. The project proper is scheduled to begin in 2007.

Ex-situ Conservation

- Exploration to collect germplasm

Rice represents the most important food crop grown in Malaysia. Genetic resources activities on the crop started with the sudden recognition of possible genetic erosion taking place in 1960s when double cropping was introduced and coupled with the rapid adoption of modern high yielding varieties. Exploration was initiated then by the Department of Agriculture to collect traditional varieties from the rice farms. This activity continued until 1969. Subsequently, with the formation of MARDI in 1970, all germplasm materials were handed over to MARDI for safe keeping at MARDI Seberang Perai research station. The initial step in genetic conservation of rice by MARDI researchers commences with the collection of traditional rice varieties and landraces from farm fields and storage bins and some wild

relatives of rice from wild habitats. A thorough survey on the distribution of wild relatives of rice throughout the country started in 1980s. Four species of rice, namely *Oryza rufipogon, O. officinalis, O. meyeriana* and *O. ridleyi* were surveyed. During the collection trip in 1989, 29 populations of *O. rufipogon*, one population of *O. officinalis* and one population of *O. meyeriana* were found. *Oryza rufipogon* is noticed to be abundant and most common in Kelantan, Seberang Prai and south Kedah. Seeds were also collected from Perak, Seberang Prai, Kedah, Terengganu, Kelantan, Sarawak and Sabah. The seeds and living plants maintained at MARDI Seberang Prai station were characterized and evaluated.

Surveys on the distribution of traditional fruits, traditional vegetables and herbs including medicinal plants in the home gardens and orchards throughout the country were carried out. The distribution and diversity of the underutilized species of fruits and these herbs were established. Sites for *in-situ* conservation for different underutilized fruit species in the country can therefore be identified. *Garcinia atroviridis* and *G. foetida* are abundant in Pahang and Terengganu, while *Nephelium ramboutan-ake* trees are concentrated in Johore. However a working relationship with tangible benefits for the farmers has to be established to make it successful.

- Seed genebank

The only seed genebank in Peninsular Malaysia is located at MARDI research station at Bertam, Seberang Perai, Penang. To date, the rice genebank consists of a total of 11,470 accessions of *O. sativa*, and 136 accessions of wild rice accessions (Table 1), of which 67% are of local origin. Besides the traditional varieties and the wild rice species, other accessions include pure line varieties, modern high yielding varieties, elite breeding lines and special types (mutants and anther derived plants). Many of the traditional varieties or landraces are no longer kept by the farmers or found in the rice farmland in the country and this situation shows clearly the cause of genetic erosion.

The seed genebank facility was financed jointly by MARDI and IPGRI (formerly known as IBPGR) and became operational in 1989. In addition to providing cold rooms for short-term, medium-term and long-term seed storage, the facility is also equipped with office space, laboratory, seed processing room, drying room and computer room.

Long-term storage: Seeds which are dried to 6-7% moisture are vacuum-sealed in aluminum foil packets each containing 18-20g seeds and stored in a deep freezer at -20^{o}C. Expected seed longevity is 30 years.

Medium-term storage: About 180-200g seeds dried to 8-9% moisture are vacuum-sealed in aluminum foil packets and kept in a cold room at 3-5^{o}C and relative humidity of 35-40%. The expected seed longevity is 15 years.

Short-term storage: Seeds with the moisture level of 10-12% are kept in brown paper envelopes and stored in a cold room at 12-15^{o}C and relative humidity of 40-45%. Expected longevity is 3-5 years.

The seed genebank has the capacity to store 25,000-30,000 accessions of seeds.

However, the traditional vegetable seeds and *ulam* (or salad-equivalent vegetable) seeds are stored in the seed genebank. Currently about 130 accessions of traditional vegetables mainly of brinjal, *Luffa,* spinach, and chili, as well as 100 accessions of *ulam* are kept in the medium-term storage (see Table 2).

In addition the seed genebank at the Agriculture Research Centre (ARC) in Tuaran, Sabah maintain 941 accessions and 18 varieties of rice as well as 38 species of vegetables (Wong, 2006). A further 1,651 accessions of rice and 29 species of vegetables are also being maintained at various research centres in Sarawak (Table 2) (Chai, 2006).

Table 2. Germplasm collection conserved at seed genebank

No.	Crops	Location	No. of accessions
1	Rice	MARDI Seberang Perai,. West Malaysia	11,470
		DOA Tuaran, Sabah	941*
		Various Centres, Sarawak	1,651
2	Traditional vegetables	MARDI Seberang Perai	130
		Specific MARDI stations	50**
		DOA Tuaran, Sabah	38***
		Various Centres, Sarawak	646****
3	*Ulam*	MARDI Seberang Seberang Perai	100

* 18 varieties of rice are also conserved
** total for chilli and tomato (non-traditional vegetables)
*** number of species
**** encompassing 29 species

- Field genebank

Conservation of tropical fruits has been given special emphasis. A sizeable germplasm collection of fruits are being maintained in MARDI as well as in other agencies (Table 3). Other field genebank collections involving coconut, coffee, root and tubers, medicinal plants, spices and beverages and *ulam* are also being established in MARDI research stations (Table 4).

Table 3. *Ex-situ* conservation of indigenous fruit species maintained at various institutions in Malaysia (Field genebanks/arboretum)

Institutions	Location (Field genebank/arboretum)	No. of species	No. of accessions
MARDI	Various stations	165	3,757*
DOA, Pen.	Serdang	17	1,000
Malaysia	Hulu Paka	110	235
DOA, Sabah	Various Agriculture Research Stations	204	n.a
DOA, Sarawak	Bintulu Agriculture Park	48	60
DOA, Sarawak	Betong Layar Station	54	n.a.
FRIM	Kepong	100	816
UPM	Serdang	36	239
UKM	Bangi	38	na
UM	Rimba Ilmu Petaling Jaya	71	207

*: Inclusive of over 1,000 accessions of traditional cultivar and land races involving 14 major fruit species

Table 4: Germplasm collection conserved in MARDI field genebank

No.	Crops	Location (Research Station)	No. of accessions
1	Coconut	Hilir Perak	45
2	Coffee	Kluang	425
3	Roots and tubers	Serdang	800*
4	Medicinal plants	Cameron Highlands Jerangau	62 3,377
5	Spices and beverages	Kluang	300
6	*Ulam*	Jerangau Seberang Perai	78 60

* Estimates

As in the above tables illustrated, collections of fruits accessions are more prominent in terms of the number of accessions collected with the next substantial collections involves medicinal plants. The collection of medicinal plants in MARDI which currently encompasses 275 species and other herbs are expected to expand further in the future with more importance being placed to it. Already the Dept. of Agriculture Sarawak has recently

established over 200 accessions of live herbal plant collections (Chai, 2006).

Utilization of Genetic Resources

Many of the wild or semi-wild species of cultivated crops possess unique and outstanding characteristics. An important activity in the management of PGRFA is on their characterization and evaluation. Researches on some wild species of rice have shown that they are resistant to major pests and diseases. The appropriate lines have been utilized in rice resistant breeding programme. *Oryza rufipogon* collected at MARDI Bumbung Lima was tested in a cross which resulted in a selection line (RU2242-2-2-1) which shows resistance to tungro virus diseases and possesses high tillering characteristics (Abdullah, 1991).

Characterization and evaluation of accessions involving under-utilised fruits such as *pulasan* (*Nephellium ramboutan-ake*) and *kuini* (*Mangifera odorata*) have resulted in the identification of 2 elite accessions within each of them. These selected accessions have high total soluble solids content (Salma, 2005). Also the assessment of the proximate contents, vitamins and minerals of over 240 accessions representing 70 underutilised fruit species showed some accessions of sour and sweet/sour *binjai* (*Mangifera caesia*) have high vitamin C content whereas *mangga telor* and *mangga bemban* (both Mangifera sp.) high vitamin A. Antioxidant activity determined on about 150 accessions representing 51 species showed some accessions of sweet/sour *binjai, kuini* and *belimbing buluh* possess superior characteristics (Salma, 2005).

Community-Driven Utilization of Crop Diversity

There are clear instances within Peninsular Malaysia, Sabah and Sarawak where agricultural utilization by the rural community themselves could have contributed to the continued sustainability of the species in a particular area. Despite minimal technical and advisory assistance from the relevant agencies the community is able to sustain the present activities and maintain it for the future generations to come. This means an *in-situ* but at the same time on-farm approach of conserving the crop species has been indirectly being pursued by the local community in that particular locality. The following examples give a brief account of such specific cases involving domesticated and semi-wild species of crop plants:

(i). Banana cv. *Pisang Kapas* (*Musa* sp.)

'Pisang Kapas' is a cooking or processing type of banana. It is considered not a popular variety if comparison is made to the more popular desert varieties such as *'Pisang*

Berangan' and *'Pisang Mas'* or even the processing variety such as '*Pisang Nipah'* and *'Pisang Tanduk'*. Substantial acreage to the extent of about few hundred acres of 'Pisang Kapas' is found only in the Batu Pahat district of Peninsular Malaysia and not in other places. They are planted along stretches of the river bank reserves, mixed with other banana varieties on the farmer's land or sometimes intercrop with immature oil palm.

The local communities here have been utilizing the variety for the production of 'pisang salai', a product derived after grilling the flesh of the ripe fruits on fire. The product is only peculiar to the area. The activities have existed a long time ago and are still actively going on.

Banana 'dealers' will initially collect the mature bunches from the field and send it to the '*pisang salai'* operators. An interesting observation is that although the mature bunches fetches about RM0.25 per kg which is considered the lowest price ever for any type of banana (as compared to RM0.40 per kg for *'Pisang Nipah'*, RM0.80 for *'Pisang Berangan'* and RM1.00 for *'Pisang Tanduk'*) the farmers continue to produce the fruits. Further analysis of the activities showed that the final product is mainly marketed within the surrounding areas especially at the Parit Raja and Ayer Hitam town centres. The latter is a popular stop-over destination for local and overseas tourists coming to and from Singapore.

To-date no proper socio-economic analysis on the viability of this home-based industry has been carried out. However interviews with a number of stall owners at those town centers mentioned earlier do indicate that there are good demands for the product.

(ii). *Mangifera caesia* (Binjai)

This fruit species is indigenous to this region. Together with the commercial mangoes, *Mangifera indica*, they belong to the family Anacardiaceae. Binjai trees are found scattered in almost all parts of the country but more plantings are observed in Negeri Sembilan, Malacca and Perak in Peninsular Malaysia. The trees are planted around the homes and along roadsides. The fruits are consumed fresh especially the sweeter types. The sour fruits are ground with chilly and prawn paste to form a palatable delicacy and consumed with rice.

Generally the species have two periods of fruiting. The first one occurred during the months of June-July while the other is from November-December. It is interesting to note that although some fruits are available at many of the town markets; substantial quantities of the fruits are displayed and sold during those months at the roadside fruit stalls in Alor Gajah district of Peninsular Malaysia. This activity existed a long time ago and this indicates there is already good linkage between the small growers (despite having few trees around their homes), fruit 'dealers' and stall owners in the area. There is no involvement of any government extension and marketing agency in the course of their production and marketing of the produce.

(iii). *Mangifera odorata* (Kuini), *M. laurina* (Mangga air) and *M. pajang* (Bambangan)

These three Mangifera species are commonly found in Sabah. Kuini is widespread throughout Malaysia whereas the last named species is endemic to Borneo Island. Many of their plantings can be found in the Kota Belud, Tuaran and Papar area in Sabah. These areas are located on the west coast of Sabah. However, in Kota Belud, many diverse forms of Mangifera plantings in the area are found on the alluvial plains of the Tempasuk River. Between the three mangifera species, kuini are the most common followed by mangga air while bambangan appear to be more scattered.

The trees are mainly planted around the houses and near the roadside with very few stands along the fringes of the forests. To-date there is no organized planting of the crop in the area. Initial studies showed that many of the plantings are very old and younger plantings have also been established especially near to the houses. Older trees have also been seen planted near and in the vicinity of the cemeteries indicating perhaps a cultural link to the use of the species concerned.

The main economic crop within the area is rice but these Mangifera species are supplementing the household income of many members of the community. The fruits are sold mainly for fresh consumption at the "Tamu" or Sunday market as well as at "Pasar Tani" markets. In general there is no assistance rendered by any of the state authorities to cater specifically to the needs of these crops.

(iv). Dimocarpus longan subsp. malesianus (Isau group)

Dimocarpus longan subsp. *malesianus* is commonly found throughout Peninsular Malaysia, Sabah and Sarawak. Three different forms of the species from Sarawak can be distinguished from the ones in the Peninsular Malaysia (Rukayah, 1999). The local community in Sarawak recognized them as '*isau'*, '*sau*' and *'kakus'*. The most distinct feature of these fruits compared with the ordinary longan is that their skins has protruded, soft and blunt spots. Many regard these fruits as typical longan unique to Sarawak. They are almost solely found along the upper reaches of the banks of the Rajang River and its tributaries covering the Kanowit, Song, Kapit and Belaga districts. They therefore thrive well on the alluvial deposits of river banks.

The *'isau'* or green longan has rounded fruits whereas 'sau' are oblong. The '*kakus'* also produce rounded fruits but upon ripening the skin changes to brownish whereas it remains green for *'isau.*' '*Isau*' are more popular as it has thicker arils and are generally sweeter with higher calcium, vitamin C and mineral contents.

Traditionally the farmers have been using seeds as planting materials. Due to its out breeding nature there are great variation in yield and fruit characteristic. *Isau* trees can grow

tall reaching a height of 20 m and it take about ten to twelve years to bear fruits. Mature trees have been recorded to yield 200 kg of fruit per season. The fruit commands a high market price. The normal fruiting season is from March to April.

(v). *Durio kutejensis* (*Durian nyekak*)

This species of *durian* can be found in Sabah and Sarawak. It is mainly found growing wild in mixed dipterocarp forests in the Bintulu and Miri divisions in Sarawak. Its fruit is popular amongst the local populace. The tree is small to medium in size having a height of 12-20 m. The tree has a close and low branching habit giving it a dense crown that is pyramidal in shape.

A mature tree may carry up to 500 fruits per season. *Nyekak* generally flowers and ripens about a month after the common *durian, Durio zibethinus*. Its flowers are deep red in colour and produce a smell. Its fruits are medium size weighing on average 1 kg per fruit. Its thorns are short and slightly bent. Another good feature is its seeds are relatively small and often flat and thin. The flesh is sweet, starchy and drier than the ordinary durians.

Nyekak has a long history in cultivation. They are mainly planted from seeds. Over the years farmers are known to select and identify apparently superior individuals. Some individuals have in fact started to systematically plant them although at small acreages.

Genebank Information System

At present database for rice genebank maintained at MARDI called Rice Genebank Information System (RGBIS) (Abdullah, 2003) have been developed. It was a relational database developed using Microsoft Access which is actually a modified version of MARDIGEN developed earlier using Dbase III that followed the format of International Rice Research Institute (IRRI) genebank. The RGBIS can facilitate two subsystems namely on documentation of the accessions and seed management. Information on seeds management includes quantity of seeds stored, tray number, age, tested date, germination rate, etc. The information helps the genebank manager to easily locate and track the accession in the genebank.

The RGBIS is currently under review with the availability of funding from Global Crop Diversity Trust. With the advantages and added features of International Crop Information System (ICIS) the rice genebank databases would be deployed for incorporation into the system. This project is again carried out with the collaboration of IRRI. The core of ICIS is it is a relational database structure that store data on plant genetic resources, pedigrees, field and laboratory evaluations, and auxiliary data on locations, institutions and people. It is expected

that with the project completion, rice genebank information in both MARDI and IRRI could be accessible via www (World Wide Web) (Tosiah and Mohd. Shukor, 2005).

Information pertaining to other crops beside rice is being developed by MARDI under the Agrobiodiversity Information System project (Tosiah, *et. al.* 2006).

Conclusion

There exists a wealth of plant genetic resources in Malaysian flora. Considerable efforts have been initiated to conserve many of the PGFRA. Extensive conservation of genetic accessions has been established in field genebanks for some of the major fruit types. Seed genebanks have been established for the conservation of rice and other orthodox-seed type of crops. Despite the availability of designated forest land gazetted as protected areas, there is a need for more concerted effort to collect and conserve more crop species encompassing wild accessions, land races and adapted ecotypes before they are lost. Emphasis in collecting a wider range of diversity within species should be attempted. There is also a need for more effort on the characterization and evaluation of PGRFA. This will allow their potential to be realized before further utilization can be effected.

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The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources
67-72

Conservation and Utilization of Plant Genetic Resources in Myanmar

By

Thaingi Swe[1]

Introduction

Plant genetic resources (PGR) are the most valuable and essential basic raw materials to meet the current and future need of crop improvement programmes. A wider genetic base assume priority in plant breeding research aimed at developing new varieties for increased crop production. PGR for food and agriculture are a reservoir of genetic adaptability, which acts as a buffer against environmental changes and economic challenges. The erosion of these resources results in a severe threat to the world's long term food security. The fundamental objective of genetic resources conservation is the maintenance of genetic diversity within each of the species. In general PGR include all species which contribute to people by providing food, medicine, feed for domestic animals, fiber, clothing, shelter, and other use. PGRs are our heritage which needs conservation for posterity.

For a primarily agriculture-based country like Myanmar, the conservation and utilization of PGR is important for economic development and food security. Our country has tremendous potential on agriculture because of its great ethnic and geographic diversity. Myanmar is home to many tropical crops species, genetic diversity is very abundant. The agriculture sector plays a major role in the economy of Myanmar. PRG is one of the major components for the enhancement of agricultural programme. The appropriate conservation and utilization of PGR is one of the key elements which can greatly contribute to the success of agriculture development and food security in Myanmar.

PGR Management in Myanmar Background

Myanmar had recognized the value of PGR even before modern agricultural technology

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was introduced. Orchards, vegetables, and medicinal plants gardens dot the countryside. The central Agriculture Research Institute, the former CARI, now, the Department of Agricultural Research (DAR) in Yezin which is the agency primarily tasking to look after Myanmar's PGR, colleting and conserving PGR have started long before established Seed banks, with more than 3000 accession of local rice varieties collected and maintained. In situ conservation of mango and medicinal plants was initiated in 1980. Systematic PGR management began after the establishment of the Seed Bank at CARI / DAR in 1987 with the support of the Japan International Cooperation Agency (JICA). Technical cooperation between Myanmar and Japan for PGR management was launched in 1997. Similarly the horticultural crops have collected and maintained in Vegetables and Fruits Research and Development Centre (VFRDC) since 1987. The VFRDC was established in 1986 and donated by JICA. The vegetables and Fruits germplasm collection began in 1998 and was supported by F.A.O.

PGR management system of Myanmar adopted the following steps:

(1) Exploration,
(2) Classification
(3) Collection
(4) Multiplication
(5) Rejuvenation
(6) Characterization
(7) Evaluation
(8) Conservation
(9) Data management
(10) Utilization

Objectives

The VFRDC has the following objectives dealing with PGR in Mayanmar. They are:

(1) To establish the PGR management system in Myanmar.

(2) To conserve and characterize vegetable and fruits collected from different regions of Myanmar.

(3) To select the promising varieties for farmer

(4) To upgrade the knowledge and technologies on PGR management of local scientists, and

(5) To exchange PGR and related information in national and global scale.

PGR Conservation Priorities

The Seed Bank is placed under DAR conserves crop species in line with national economic policies. Therefore, collection and conservation of rice, oil seed crops, and food legumes are given priority.

The VFRDC conserves horticulture species in line with national economic policies. Conservation of medicinal orchids, kitchen crops, such as ginger, chili, onion, potato, and bio-diesel crops (Jatropha).

Exploration, Collection and Introduction Activities

At present, totally, 13,046 accessions for 28 crop species are collected by *ex situ* collection in Seed Bank conservation under the DAR.

From 1998 to 2006, in the implementation of germplasm collection, 1,155 accessions of vegetables and 340 accessions of fruits have been collected and conserved in VFRDC.

Multiplication, Characterization and Evaluation Activities

Characterization of germplasm based on morphological characters and molecular markers. Morphological characters are used for basic characterization according to the descriptors. This technique is capable to reach a high sample through put and this information is of high interest for users of genetic diversity of PGR. Molecular markers provide an extremely useful technology for conservation and use of PGR. VFRDC is working on the biodiversity project collaboration with Tsukuba University, Japan. All results suggest that Myanmar germplasm belong to a rich genetic variation at molecular level. Up to now, 896 accessions of vegetables and 241 accessions of fruits (especially in fruit quality) have been characterized and evaluated in VFRDC. These research activities have been conducted in VFRDC, Myanmar Agriculture Service (MAS) and its related horticultural farms. There are 61 horticultural farms are administrated under MAS, Ministry of Agriculture and Irrigation (MOAI). Among them, 16 research farms from different ecological regions of Myanmar are collaborated with VFRDC for PGR research. Some of the varieties, such as potato and strawberry cannot multiply in VFRDC because of temperature and climate. Therefore, we conserve them by tissue culture and sent to other suitable regions for multiplication and locality tests. VFRDC also cooperates with Yezin Agricultural University, DAR, and University of Yangon to conduct some of PGR research activities.

Conservation and Utilization

The objective of the conservation is to preserve PGR safely without loss in viability, genetic integrity while maintaining original spectrum of variability. VFRDC has three types of cold storage facilities: 0-5ºC, 5-10ºC, and 10-15ºC room temperature without R.H controller. The cold storage facilities of VFRDC are still required for germplasm conservation. The collected seeds were packed in aluminum foil bags and stored in above storage rooms. Most of fruit crops were conserved in field genebank. The seed bank has three types of conservation facilities: short-term, medium, and long-term storage rooms. Seeds of active collection in polyethylene bottles are stored in short-term facilities maintained at 5-10ºC with 30-40% RH. Seeds of base collection are vaccum packed in aluminum foil bags and stored in medium to long-term facilities maintained at -5ºC with 30-40% RH.

Crop germplasm conserved in the Seed Bank & VFRDC are supplied upon request. Seed distribution for other government agencies within Myanmar has been done after accomplishing the official request form. In the case of international requests, seeds are supplied with Material Transfer Agreement (MTA). Totally, 680 crop germplasm accessions of Seed Bank, Dept. of agricultural Research (DAR) have been distributed within Myanmar and 4,555 accessions have been supplied to the International Rice Research Institute (IRRI), the National Institute of Agro-biological sciences (NIAS-JAPAN), Kyushu University of Japan, and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Totally, 290 accessions of horticultural crop germplasm have been distributed from VFRDC to abroad (Okayama University, NIAS-JAPAN, JICA) with Material Transfer Agreement.

Documentation and data management forms and formats have been unified and database construction has also been completed in DAR, Yezin. Within DAR, a Local Area Network (LAN) system has been established for smooth data flow. Documentation and data management of PGR in VFRDC has still required and upgraded.

Pre-breeding activities should be carried out to assure better use PGR and to link crop breeding programme. Pre-breeding activities are expected to include critical evaluation of crop germplasm for specific traits such as bacterial blight resistance, bacterial wilt resistance, fruit and shoot borer (FSB) resistance, grain quality, fruits quality, and so on. The promising varieties of some vegetables (F_1 Melon Tomato, Chili, Egg plant, Okra, YLB, Cucumber, Pumpkin, and etc.) were selected and hybridized by using PGR in long term breeding programme of VFRDC.

Conclusion

Although PGR is very abundant, the adoption rate of modern varieties and technology continue to increase to feed 54 million people in Myanmar. So, many traditional varieties and landraces would give way to modern varieties. In addition, the destruction of habitats of wild species and related genera because of rapid urbanization is predictable in near future as the population increase. In order to prevent the permanent loss of genetic diversity in many tropical crop species, PGR management, conservation and utilization research activities have become critical issues for Myanmar agriculture improvement. There have several thousand materials of germplasm collected from domestic and introduced origins have been conserved in seedbank and other facilities but breeding materials are still of scarceness. A strong national programme on plant genetic resources conservation and utilization also needs to be developed by commitment and supported from various national and international state holders. In order to promote PGR management and research activities, the numbers of members with suitable qualification need to be increased. Therefore, training in PGR management at the national and international levels is apparently essential. Myanmar recognizes the value of PGR for both present and the future. Within our capacity, Myanmar is working to value and conserve PGR as a national heritage. Therefore, Myanmar needs all possible assistance, financial or in kind, technical backstopping, collaborative research, information and public awareness materials, and working relationship with international scientists in PGR management. Through the exchange of PGR and PGR information, Myanmar would be able to strengthen its goodwill with partner countries.

Table 1. Germplasm conservation of vegetables in VFRDC

No.	Crop	No. of accession	Conservation
1	Solanaceae	341	Field & storage
2	Cucurbitaceae	261	Field & storage
3	Cruciferae	210	Field & storage
4	Fabaceae	95	Field & storage
5	Zingiberaceae	64	Field & storage
6	Others (eg. Onion, Okra, etc.)	184	Field & storage
	Total	**1155**	

Table 2. Germplasm conservation of fruits in VFRDC

No.	Crop	No. of accession	Conservation
1	Mangoes	182	Field
2	Dragon Fruit	3	Field
3	Grape	8	Field
4	Durian	2	Field
5	Mangosteen	3	Field
6	Pummelo	6	Field
7	Banana	35	*In vitro* and field
8	Orange	11	*In vitro* and field
9	Pineapple	6	*In vitro* and field
10	Strawberry	3	*In vitro* and field
11	Potato	32	*In vitro* and field
12	Custard Apple	1	Field
13	Sour soap	1	Field
14	Marian	2	Field
15	Lychee	1	Field
16	Star Fruit	1	Field
17	Java Apple	3	Field
18	Sapodilla	1	Field
19	Guava	2	Field
20	Indian jujube	1	Field
21	Hog Plum	1	Field
22	Kepundung	1	Field
23	Bael Fruit	1	Field
24	Jack Fruit	3	Field
25	Rambutan	1	Field
26	Djenkol Bean	1	Field
27	Dwarf Coconut	1	Field
28	Pome granate	3	Field
29	Star Apple	1	Field
30	Kadibathi	1	Field
31	Papaya	3	Field
32	Others (Orchids, Jatropha, Aloe, etc.)	19	*In vitro* and field
	Total	**340**	

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Evaluation of Myanmar Rice Germplasms for Resistance to Bacterial Blight

By

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Abstract

Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* from four major rice growing divisions of Myanmar was investigated. One hundred and thirty two isolates: 28 isolates from Ayeyarwady Division, 17 from Yangon Division, 42 from Bago Division and 45 from Mandalay Division, collected during 2004 and 2005 rice growing seasons were very diverse in virulence on 12 near-isogenic lines each of which carrying a specific resistance gene, *Xa 1*, *Xa 2*, *Xa 3*, *Xa 4*, *xa 5*, *Xa 7*, *xa 8*, *Xa 10*, *Xa 11*, *xa 13*, *Xa 14*, or *Xa 21*. The isolates were classified into 19 races based on their virulence. Among 19 races, Race 17 which consisted of 21.97% of the test isolates, was not only the most predominant race but also prevalent in 4 major rice growing divisions of Myanmar. It was also virulent on most of the differentials except IRBB 13 and IRBB 21. Race 7 and Race 11 fell into the second and third position in terms of predominance, respectively. However, Race 11 was found in four major rice growing divisions except Yangon Division, and Race 7 was detected in Bago and Mandalay Divisions only. Similarly, most of the other races found in one or two divisions were not detected in other divisions. The most predominant race in one division was also different from that of other divisions.

Pathogenic diversity of four selected isolates collected from Hmawbi, Bogale, Paukkhaung and Kyaukse was confirmed by computer-assisted image analysis method in quantifying disease severity. The virulence of each of the four isolates on three near-isogenic lines, IRBB 3, IRBB 7 and IRBB 13 each carrying a specific resistance gene *Xa 3*, *Xa 7*, and *xa 13*, respectively, was qualitatively different from that of other isolates. One hundred and thirty-four Myanmar rice germplasms obtained from Seed Bank, Department of Agricultural

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Research, Yezin, were evaluated for their resistance to the representative isolates of four races. The test rice germplasms were divided into four groups, Group A, B, C and D, based on their reaction to four isolates. Two rice germplasms namely Mya Wut Yi and Talay were belonged to Group D and resistant to three representative isolates of the three most predominant races detected in four major rice growing divisions of Myanmar.

Introduction

Myanmar is an agricultural country, and agricultural sector is the backbone of its economy. Rice is the principal human food resource and primary foreign exchange earner of Myanmar. It is being widely grown throughout the country occupying an area of about 6.0 million ha and sharing about 50% of the total crop sown area. Based on isozyme polymorphism, Nakagahra (1978, 1987) proposed that the center of genetic diversity of rice was situated in an area consisting of northern part of Myanmar, Laos and Yunnan Province of China. Seed bank was established in Yezin, Myanmar in 1990. It is the national centre of plant genetic resources in the country which has been collecting, introducing, characterizing, evaluating and conserving diverse plant genetic resources. One of the main objectives of Seed Bank is to ensure the effective utilization of germplasms in breeding programs so as to contribute to agricultural development in Myanmar (CARI, 2001). Rice, its wild relatives and some major crops have mainly been focused and now more attention is paid to minor crops and wild plants. Rice diseases, insect pests and weeds are major constraints for increasing productivity of rice. Approximately 74 pathogens are associated with rice and 16 diseases are economically important (Roger *et al.* 1991). Among them, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the major constraints in rice production. Yield losses in severely infected field ranged from 20 to 30%, and may on rare occasions be up to 50% (Ou 1985).

Some chemicals have been developed to control the disease. However, none of them has been fully effective under very severe conditions (Noda *et al.* 1996). As chemical control may have an adverse effect on the environment and is costly, growing resistant cultivars is the most safe and feasible control measure against the disease (Ezuka and Kaku 2000). To develop resistant varieties by hybridization, identification of resistant donors is the first step. In the early stage of research on bacterial blight of rice, specific differentiation of virulence in the pathogen, *Xanthomonas oryzae* pv. *oryzae*, was not known. So the resistance of rice cultivars was evaluated simply by inoculating a single bacterial isolate. Nowadays, the existence of races in *X. oryzae* pv. *oryzae* is an established fact that is accepted by all the researchers working on bacterial blight of rice (Ezuka and Kaku 2000).

To control bacterial blight of rice based on host resistance, determination on pathogenic diversity of *X. oryzae* pv. *oryzae* is a prerequisite. Evaluation of resistance in rice germplasms and incorporation of the resistant genes into improved varieties may then be investigated. The present study was, therefore, conducted with the following objectives:

1. to determine the pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* from major rice growing divisions of Myanmar,
2. to examine the distribution of races of *X. oryzae* pv. *oryzae* in different rice growing regions,
3. to identify Myanmar rice germplasms resistant to different races of *X. oryzae* pv. *oryzae* and
4. to provide resistant donors for rice breeding program for bacterial blight resistance.

Materials and Methods

Determination on Pathogenic Diversity of *Xanthomonas oryzae* pv. *oryzae* from Major Rice Growing Divisions of Myanmar

One hundred and thirty-two isolates of *X. oryzae* pv. *oryzae* collected from 27 townships of 4 major rice growing divisions of Myanmar (Figure 1) during 2004 and 2005 rice growing seasons were evaluated for their virulence on differential varieties. Diseased leaf samples of bacterial blight of rice were collected from diseased fields of different locations. Isolation of the pathogen was done as soon as possible after the leaf samples were collected. Proposed international differential rice varieties for *X. oryzae* pv. *oryzae* (Ogawa 1993) namely IRBB 1, IRBB 2, IRBB 3, IRBB 4, IRBB 5, IRBB 7,IRBB 8, IRBB 10, IRBB 11, IRBB 13, IRBB 14 and IRBB 21, each carrying a specific resistance gene *Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa14*, and *Xa21*, respectively, were used for determination of pathogenic diversity of *X. oryzae* pv. *oryzae*. TN 1 was tested as a checked variety.

Thirty-day old seedlings of each test variety were transplanted individually in one row plot at 30 x 30 cm spacing. The plants were grown up to panicle initiation to booting stage in accordance with the normal cultivation practices. The flag leaves or the uppermost fully expanded leaves of five tillers were inoculated by clipping method at 90 days after sowing. The experiment was laid out on a strip plot design with three replications. Disease recording was done 21 days after inoculation (DAI) according to the IRRI's Standard Evaluation System for Rice (IRRI, 1988). Percent leaf area infected on inoculated leaves was visually estimated. Disease reactions of the rice varieties to the bacterial isolates were determined based on

disease scores. The variety was ranked as resistance (R) if the disease score was 3 or less and susceptible (S) if it was more than 3.

Confirmation on Pathogenic Diversity *of Xanthomonas oryzae* pv*. oryzae*

Pathogenic diversity of *X. oryzae* pv. *oryzae* was confirmed by computer-assisted image analysis method in quantifying disease severity. Four isolates of *X. oryzae* pv. *oryzae* designated as 04107, 04172, 0505 and 0530 which were representative isolates of Race 14, 11, 17, and 7, respectively, were tested to confirm the pathogenic diversity of the pathogen.

Three near-isogenic lines namely IRBB 3, IRBB 7 and IRBB 13 each carrying a specific resistance gene *Xa 3*, *Xa 7*, and *xa 13*, respectively, were selected as differentials based on the results of previous experiment. IRBB 21 and TN 1 were used as resistant check and susceptible check variety, respectively. The plants were grown up to 90 days after sowing. The same inoculation method was again employed here as in the previous experiment. The experiment was laid out on a strip plot design with four replications. Disease severity (percent leaf area infected) was measured 21 days after inoculation by computer-assisted image analysis method.

Total leaf area and infected leaf area of the inoculated leaves were measured by computer-assisted image analysis method and percent leaf area infected was calculated. The inoculated leaves were detached from the plant and made flat by applying pressure with a hot iron. The leaves were then mounted on white cards with 30 cm x 22 cm area, scanned and saved as digital images with 1.2 million pixels resolution. To determine the percentage of leaf area infected, colour threshold method was applied using SigmaScan Pro 5.0 (SPSS Inc.) image analysis software. Image of the inoculated leaves appeared on the image window of SigmaScan Pro 5.0 was duplicated. An overlay in red colour was applied to defined measurement parameters for the infected portions. Colour threshold method was applied comparing with the original image of the leaves. Then infected leaf area was measured using the Measurements / Measure Objects tab. The area of the infected portions was obtained on the data work sheet (Plate 1 A, B, C, D & E). To measure the total leaf area, the original image of the leaves was duplicated again, and the entire portion of the duplicated image of the leaves was determined using fill mode as mentioned above. Total leaf area was shown on another data work sheet (Plate 1 F).

Evaluation of Myanmar Rice Germplasms for Resistance to Different Races of *Xanthomonas oryzae* pv. *oryzae*

Totally, 134 indigenous rice germplasms from 14 states and divisions of Myanmar, obtained from Seed Bank, Department of Agricultural Research, Yezin, were tested for resistance to different races of *X. oryzae* pv. *oryzae*. The plants were grown up to 90 days after sowing.

The representative isolates of 4 races, Race 14, 11, 17, and 7, of *X. oryzae* pv. *oryzae* designated as 04107, 04172, 0505, and 0530, respectively, were tested for their virulence on indigenous rice germplasms. Inoculum preparation for each isolate and inoculation to the test germplasms were carried out as described in the previous experiment. The experiment was laid out on a strip plot design with four replications. Evaluation for resistance of each germplasm was done at 21 DAI according to Standard Evaluation System for Rice (IRRI 1988).

Results and Discussion

Pathogenic Diversity of *Xanthomonas oryzae* pv. *oryzae* in 4 Major Rice Growing Divisions of Myanmar

One hundred and thirty-two isolates of *X. oryzae* pv. *oryzae* collected from four major rice growing divisions of Myanmar: 28 isolates from Ayeyarwady Division, 17 from Yangon Division, 42 from Bago Division and 45 from Mandalay Division, were very diverse in virulence on differentials, 12 near-isogenic lines. The bacterial isolates were classified into 19 races based on their virulence on the differentials. All isolates tested were virulent on TN 1 but avirulent on IRBB 21. Virulence of these isolates on other differentials varied with the combination of the differentials and races (Table 1).

Table 1. Pathogenic diversity[x] of 132 isolates of *X. oryzae* pv. *oryzae* from 4 major rice growing divisions of Myanmar on 12 near-isogenic lines

Races	No. of isolates	Differential varieties													Isolates (%)
		IRBB 21	IRBB 7	IRBB 13	IRBB 3	IRBB 4	IRBB 1	IRBB 5	IRBB 14	IRBB 10	IRBB 2	IRBB 8	IRBB 11	TN 1	
1	11	R	R	R	R	R	R	R	R	R	R	R	R	S	8.33
2	1	R	R	R	R	R	R	R	R	R	R	S	S	S	0.76
3	1	R	R	R	R	R	R	R	R	S	R	R	S	S	0.76
4	1	R	R	S	R	R	R	R	R	R	R	R	S	S	0.76
5	1	R	R	S	R	R	R	R	R	R	R	S	S	S	0.76
6	1	R	R	S	R	R	R	R	R	R	S	S	S	S	0.76
7	24	R	R	S	R	R	S	S	S	S	S	S	S	S	18.18
8	11	R	R	S	R	S	S	S	S	S	S	S	S	S	8.33
9	1	R	R	S	R	S	S	S	S	S	S	S	R	S	0.76
10	2	R	R	S	S	R	S	S	S	S	S	S	S	S	1.52
11	16	R	R	S	S	S	S	S	S	S	S	S	S	S	12.12
12	1	R	R	S	S	S	S	S	S	S	S	S	R	S	0.76
13	4	R	S	R	R	R	S	S	S	S	S	S	S	S	3.03
14	3	R	S	R	R	S	S	S	S	S	S	S	S	S	2.27
15	2	R	S	R	S	R	S	S	S	S	S	S	R	S	1.52
16	9	R	S	R	S	R	S	S	S	S	S	S	S	S	6.82
17	29	R	S	R	S	S	S	S	S	S	S	S	S	S	21.97
18	3	R	S	S	R	R	S	S	S	S	S	S	S	S	2.27
19	11	R	S	S	S	S	S	S	S	S	S	S	S	S	8.33

[x]R = Resistant, 0 to 12 % leaf area infected
S = Susceptible, 13 to 100 % leaf area infected

Race Composition of the Test Isolates

Of 132 isolates of *X. oryzae* pv. *oryzae* tested in this study, 11 were classified as Race 1 and this race was virulent only on TN1 but a virulent on other differentials. On the other hand, another 11 isolates belonged to Race 19; it was virulent on all of the differentials except IRBB 21. Among 19 races, Race 17 which accounted for 21.97% of the test isolates was the most predominant race. This race was also virulent on most of the differentials except IRBB 13 and IRBB 21. Race 7 was the second-most predominant race; it comprised 18.18% of the test isolates. The virulence of Race 7 and Race 8 on the differentials was similar to each other except the virulence on IRBB 4. Race 11 which contained 12.12% of the test isolates was the third-most predominant race. This race was also avirulent only on two differentials, IRBB 7 and IRBB 21. Most of the other races consisted of only one or two isolates (Table 1). Thus, the three most predominant races, i.e. Race 7, 11, and 17, occupied 52.57% of the test isolates, and these races were virulent on 8 to 10 differentials each carrying a specific resistance gene. In other words, most of the resistant genes tested were defeated by the predominant races. Therefore, most of the currently available sources of major resistance genes are unlikely to provide effective control against the predominant races which currently exist in four major rice growing divisions of Myanmar.

Distribution of Races in Four Major Rice Growing Divisions

Among the races classified in this study, Race 17 was the most predominant one. It was found in all of the four major rice growing divisions, 3.03%, 4.55%, 8.33% and 6.06% of the test isolates in Ayeyarwady, Yangon, Bago and Mandalay Divisions, respectively. Therefore, Race 17 was not only the most predominant but also prevalent race in four major rice growing divisions of Myanmar. However, the second-most predominant race, Race 7, was found in only two major rice growing divisions, Bago and Mandalay Divisions. On the other hand, the third-most predominant race, Race 11, was not detected in Yangon Division. Similarly, some of the other races detected in one or two divisions were not found in other divisions (Table 2).

Table 2. Distribution (percentage of isolates) of races of *Xanthomonas oryzae* pv. *oryzae* in different locations

Race	Location (Division)				
	Ayeyarwady	Yangon	Bago	Mandalay	Total
17	3.03	4.55	8.33	6.06	21.97
7	-[x]	-	5.30	12.89	18.18
11	7.58	-	3.79	0.76	12.12
1	6.06	-	0.76	1.52	8.33
8	-	0.76	2.27	5.30	8.33
19	2.27	3.79	2.27	-	8.33
16	-	-	5.30	1.52	6.82
13	-	-	-	3.03	3.03
14	-	0.76	-	1.52	2.27
18	-	1.52	-	0.76	2.27
10	-	-	1.52	-	1.52
15	-	-	1.52	-	1.52
2	0.76	-	-	-	0.76
3	-	-	0.76	-	0.76
4	1.76	-	-	-	0.76
5	-	0.76	-	-	0.76
6	-	-	-	0.76	0.76
9	-	0.76	-	-	0.76
12	0.76	-	-	-	0.76

[x] - Not detected

If the same race occurs in all locations, as Race 17 detected in all of the four major rice growing divisions, screening for resistance could be carried out with the common race. However, when different races occur in different locations, it makes screening more complicate. It means that screening for resistance has to be done with different races due to differences in pathogen population among locations.

Confirmation on Pathogenic Diversity *of Xanthomonas oryzae* pv*. oryzae*

Virulence of four isolates of *X. oryzae* pv. *oryzae,* 04172, 0505, 0530 and 04107 on four near isogenic lines, IRBB 3, IRBB 7, IRBB 13 and IRBB 21, and TN 1 were tested. Computer-assisted image analysis method was applied to measure disease severity. It was observed that disease severity (% leaf area infected) on the test varieties inoculated with four bacterial isolates showed highly significant differences among the varieties and among the isolates. The interaction between the varieties and the isolates was also highly significant (Table 3).

Table 3. Analysis of variance for disease severity (% leaf area infected) measured by computer-assisted image analysis method on five rice varieties inoculated with four isolates of *Xanthomonas oryzae* pv. *oryzae*

Source of variation	Degree of freedom	Sum of squares	Mean squares	Observed F
Replication	3	106.19	35.40	1.42 ns
Variety	4	45559.23	11389.81	457.72 **
Error (a)	12	298.61	24.88	-
Isolate	3	2219.58	739.86	21.95 **
Error (b)	9	303.42	33.71	-
Variety x Isolate	12	31992.51	2666.04	44.74 **
Error (c)	36	2145.45	59.60	-
Total	79	82624.98	-	-

CV (a) = 13.4%; CV (b) = 15.5%; CV (c) = 20.7%
**: significant at 1% level; ^{ns}: not significant

Zadoks and Schein (1979) indicated that interaction is the mathematical way of describing the biologic phenomenon called physiologic specialization. They stated that when there is interaction, some varieties differentiate between the pathogen isolates and likewise these pathogen isolates differentiate between the varieties. The results of the present investigation, therefore, suggested that there was a distinct differential interaction between the variety-isolate combination and proved the pathogenic diversity among the test isolates of *X. oryzae* pv. *oryzae.*

Four isolates of *X. oryzae* pv. *oryzae* were distinctly different in their pathogenicity on three near isogenic lines namely IRBB 3, IRBB7 and IRBB 13. Isolate 04172 was virulent on

IRBB 3 and IRBB 13 but avirulent on IRBB 7 whereas another isolate, 04107 was avirulent on IRBB 3 and IRBB 13 but virulent on IRBB 7. On the other hand, Isolate 0505 was virulent on IRBB 3 and IRBB 7 but avirulent on IRBB 13. However, the virulence of the isolate 0530 on the three near isogenic lines was reverse to that of isolate 0505. All of the isolates were virulent on TN 1 and avirulent on IRBB 21 (Table 4, 5, Fig 2, and Plate 2, 3). According to these results, the virulence of each isolate on the test varieties was qualitatively different from that of other isolates. This kind of differences in pathogenic capacity could be accounted for in screening and breeding programs for bacterial blight resistance.

Table 4. Disease severity (% leaf area infected) measured by computer-assisted image analysis method on five rice varieties inoculated with four isolates of *Xanthomonas oryzae* pv. *oryzae*[x].

Bacterial isolates	Rice varieties				
	IRBB 3	IRBB 7	IRBB 13	IRBB 21	TN 1
04172	58.33 a B[y]	2.38 b C	53.80 b B	5.13 a C	77.72 ab A
0505	60.10 a B	58.64 a B	7.77 c C	8.96 a C	86.36 a A
0530	10.12 b C	6.98 b C	67.63 a B	8.91 a C	84.06 ab A
04107	6.02 b C	56.39 a B	5.36 c C	7.06 a C	75.19 b A

CV (a) = 13.4 %; CV (b) = 15.5 %; CV (c) = 20.7 %
[x] Inoculated 90 days after sowing and measured 21 days after inoculation
[y] Means followed by the same letter are not significantly different at 5%LSD . Small letters indicate comparison of means in each column while capital letters indicate comparison in each row.

Table 5. Disease reactionx of five rice varieties to four isolates of Xanthomonas oryzae pv. oryzaey

Rice varieties	Bacterial isolates			
	04172	0505	0530	04107
IRBB 3	S	S	R	R
IRBB 7	R	S	R	S
IRBB 13	S	R	S	R
IRBB 21	R	R	R	R
TN 1	S	S	S	S

[x] R = Resistant (Disease score 3 or less)
S = Susceptible (Disease score more than 3)
[y] Inoculated 90 days after sowing and measured 21 days after inoculation

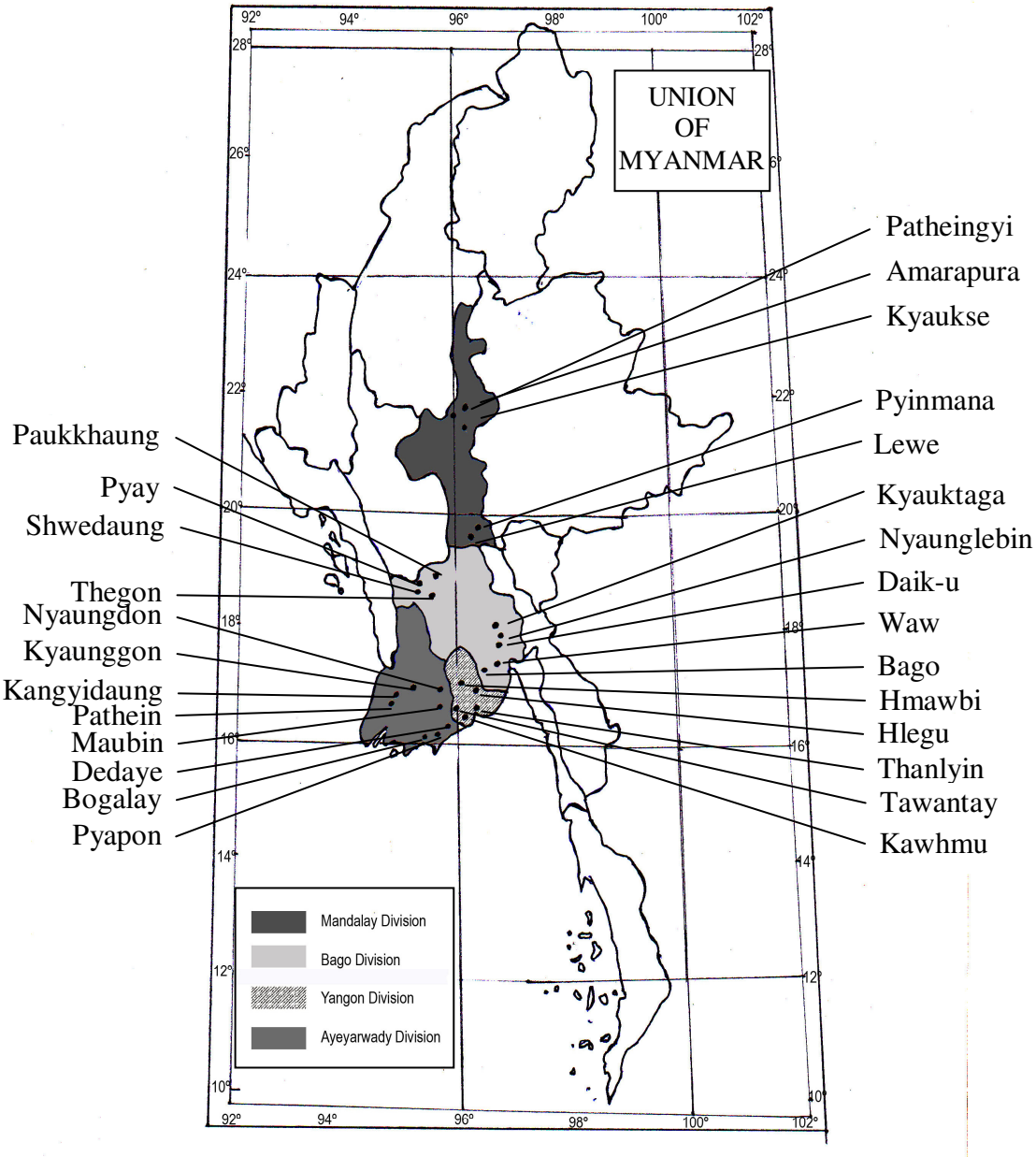

Figure 1. Collection sites (townships) of test isolates of *X. oryzae* pv. *oryzae*

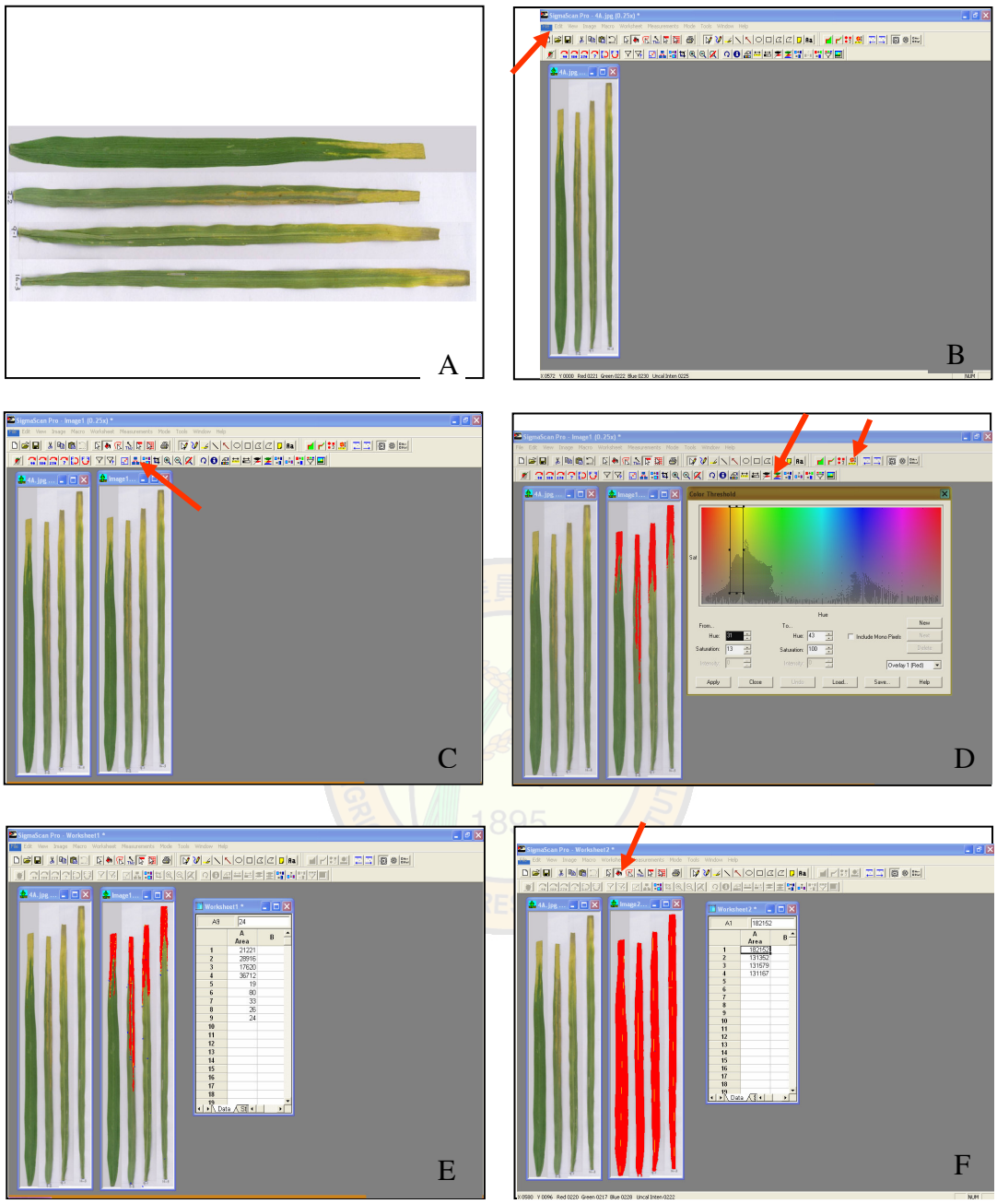

Plate 1. Measuring infected and total leaf areas of inoculated leaves by computer-assisted image analysis method

A. Inoculated leaves, on which % leaf area infected is to be measured
B. Opening the file; image of the leaves appeared on the image window of Sigma Scan Pro 5.0
C. Duplication of the image of leaves to be measured
D. Overlaying with red colour on the infected portion of the duplicated image of leaves using fill mode; colour threshold method was applied to measure the infected area
E. Measuring the infected leaf area; the area was shown on the data work sheet
F. Measuring the total leaf area; the original image of the leaves was duplicated again, the entire portion of the duplicated image of the leaves was defined using fill mode, and total leaf area was shown on another data work sheet

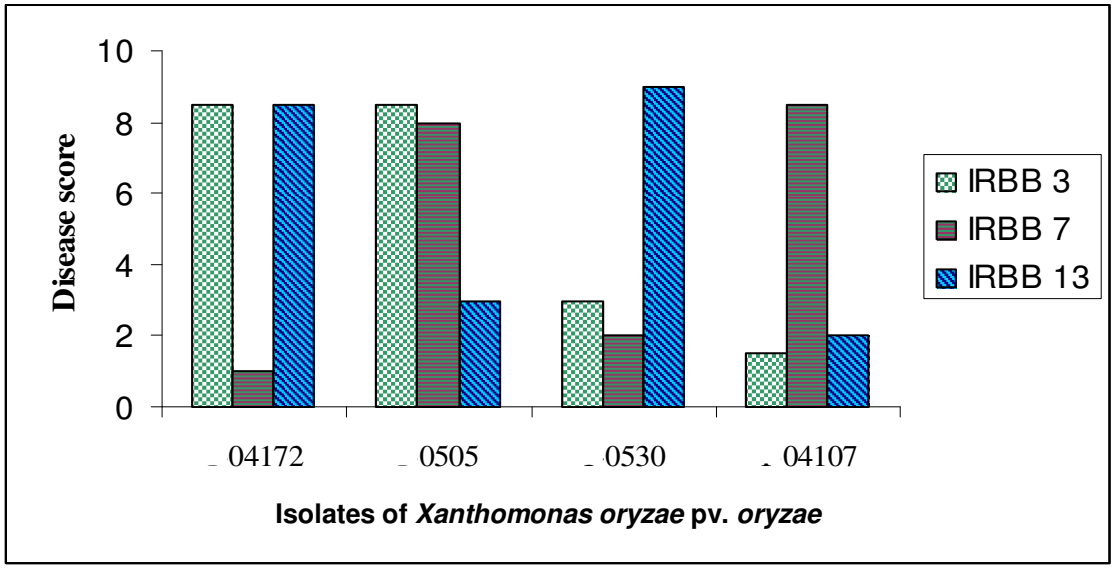

Figure 2. Virulence of four isolates of *Xanthomonas oryzae* pv. *oryzae* on three near isogenic lines

Evaluation of Myanmar Rice Germplasms for Resistance to Different Races of Xanthomonas *oryzae* pv. *oryzae*

Indigenous rice germplasms of Myanmar were evaluated for resistance to four Myanmar races of *X. oryzae* pv. *oryzae*. On the basis of reaction patterns to representative isolates of four races, 134 rice germplasms were categorized into four varietal groups, Group A, B, C and D.

Seventy-eight rice germplasms included in Group A were susceptible to all of the representative isolates of four races. In Group B, there were 50 rice germplasms and they were resistant to Race 11, but susceptible to other three races. Only 4 and 2 rice germplasms belonged to Group C and D, respectively. Four rice germplasms of Group C, Khae Saba, Van Lai Phal, Ant Baw and Baw Gyi, were resistant to two races, Race 7 and 11. Mya Wut Yi and Taylay, which belonged to Group D, were resistant to three races, Race 7, 11 and 17 (Table 6 and 7).

Plate 2. Disease reaction of IRBB 21 and TN 1 to fourisolates of *Xanthomonas oryzae* pv. *oryzae*

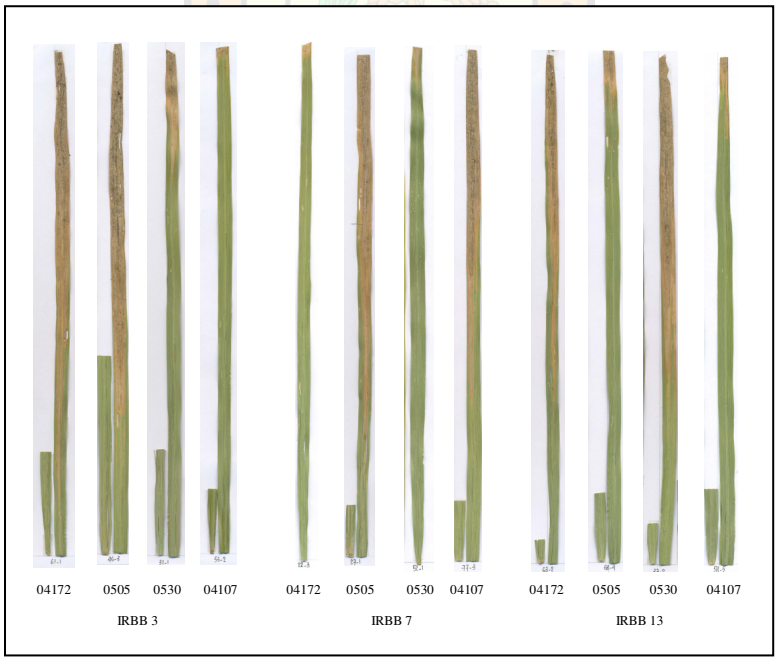

Plate 3. Disease reaction of three near-isogenic lines, IRBB 3, IRBB 7 and IRBB 13 to four isolates of *Xanthomonas oryzae* pv. *oryzae*

Table 6. Grouping of 134 Myanmar rice germplasm on the basic of reaction patterns to four isolates* of *Xanthomonas oryzae* pv. *oryzae*

Group	Varieties
A	Khao Kain, Magauk, Saba Ni, Khao Hlaing, Moe Thay, Kyaw Par Lae, Kha Yan Gyar, Chin Pa Chee, Khao Mon Moe Nae, Khao Mon Han Young, Lin Ban Chaw,Khao La Shae, Khao Pha Yaung, Bu Do Ya, Saba Khar, Khao Pae Law, Khao Maw Kyan, Ekare, Ekarin Kwa, Shwe Pyi Aye, Bu Har, Baria, Rel Beng, Dug Thin, Kalagyi, Kyaw Galay, Kari Let Yone, Lone Phyu, Yay Thu Ma, Shan Nyein, Kyun Shwe War, Nga Kauk Che, Sar Pa Si Chay, Nga Kyein Thee, Sa O Me, Ohn Ni Ma, Ya Khine Ma, Khao Saw, Khao Man Mu, Khao Kan, Let The Zaung, Pale Pyu, In Gyin Su, Kauk Latt Phyu, Shan Ma Lay, Pa Dee Pu, Pa Che Mwe Swe, A Pyo Daw Gyi, Kyauk Htu, Phwe Htaung Ni, Ye Tha, Sabani Hmwe, Ah Nauk Chaung, Yatha Lay Long Thwe, Nga Kywe Taung Pyan, Khun Na Ya Thee Dat, Nga Pya Yin, Bu Thout, San Gyi, Pin Shwe War, Kalar Htun, Sa Sar, Shwe Phyu, Thone Hnan Latt, Tha Htay Gyi, Ywet Thay, Mwe Swe, Okshit, Naung Aine, Taung Hteik Pan, Kauk Thwe Phyu, Thet Nu Bay Kyar, Nga Kyee Du Me, Let Yone, Kauk Thwe, Let Yone Gyi, Kywe Chae Manaing and Nga Yar Nga Se
B	Naung Tu, Let The Nu, Khao Lar Say, Nga Yar Gyaw, Kauk Kyi Saw, Shwe Pu, Yay Thu Ma, Epa Tine, Dawe Sein, Kala Lay, Ekare Phyu, Naga Yar, Mya Sein, Bahat Ni, Gauk Ya, Ah Nauk Thama, Khao San Haung, Khao Man Mon Tine, Khao Kyaine, Khao Kyauk Oun, Khao Mwe Naung, Khao Note Tit, Takade, Tay Lay, Shwe Bo Khun Ni, Ya Thae, Wun Htauk, Nga Sein Du Me, Shwe Chay Gyin, Paw La Khe, Thone Poke Ta Pote, Nga Kywe Phyu, Shwe Dinga, Aung Lan Daw, Pyi Daw Aye, Hnan War, Wa Ti Tun, Du Wa Pho, Ban Gauk, Man Nga Sein, Mee Kauk, Shwe Kyi Dauk, Kauk Sann, Shwe Kyi Nyo, Ngar Yar Kyaw, Baw Kyan, Ma Po Lay, Maung Nyo Sann, Balakyar and Phya Pon Thee Dat
C	Van Lai Phai, Ant Baw, Khae Saba and Baw Gyi
D	Mya Wut Yi and Talay

*: Representative isolates of 4 races, 04172, 0505, 0530, and 04107, i.e., races 11, 17, 7, and 14, respectively

Table 7. Disease reaction[x] of four groups of rice germplasms to four isolates of *Xanthomonas oryzae* pv. *oryzae*

Varietal group	No. of germplasms belong to each group	Isolates[y]			
		04172	0505	0530	04107
A	78	S	S	S	S
B	50	R	S	S	S
C	4	R	S	R	S
D	2	R	R	R	S

[x] R = resistant, 0 to 12% leaf area infected
S = susceptible, 13 to 100% leaf area infected
[y] Representative isolates of 4 races, 04172, 0505, 0530 and 04107, i.e., races 11, 17, 7, and 14, respectively

In order to develop bacterial blight resistant varieties, selection of appropriate donor parents which show broad spectrum of resistance is important for the breeding program. Thus, evaluation of introduced and indigenous rice germplasms for bacterial blight resistance is one of the primary tasks for rice genetic resources management and utilization. In the present study, 134 indigenous rice germplasms of Myanmar were evaluated for resistance to four Myanmar races of *X. oryzae* pv. *oryzae*. It was observed that, majority of the indigenous rice germplasms, i.e. 78 germplasms of Group A, were susceptible to four Myanmar races. On the other hand, 50 rice germplasms included in Group B were resistant to Race 11. This race was one of the three predominant races detected in this study and distributed in three major rice growing divisions, Ayeyarwady, Bago, and Mandalay Divisions. Therefore, these rice germplasms could be used as resistant donors in varietal improvement program for those divisions.

The discovery of pathogenic specialization led to the incorporation of different resistance genes into improved cultivars. Thus, modern breeding program paid an attention to develop rice varieties which are resistant to multiple races of *X. oryzae* pv. *oryzae*. In the present study, Khae Saba, Van Lai Phal, Ant Baw and Baw Gyi were resistant to Race 7 and 11, and Mya Wut Yi and Taylay were resistant to Race 7, 11 and 17. These three races were predominant races detected in four major rice growing divisions of Myanmar. It indicated that these six indigenous rice germplasms were potential donors for multiple race resistance in breeding program for bacterial blight in Myanmar.

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Conservation and Utilization of Plant Genetic Resources in the Philippines: Status and Directions

By

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Introduction

Plants are essential in the development of human society and serve as the key to food security, as they provide not only food, but also fiber and shelter. Conserving and using plant genetic diversity are essential to meet the world's future development needs, considering that the earth's population places enormous pressure on the environment. Before the end of the 21st century, a huge increase in the production of food and non-food commodities would be required. The wise use of plant genetic resources can help eradicate poverty, and also protect and enhance the environment.

The Country: Philippines

Geography, Area and Climate

The Philippines has a total land area of 115,830 square miles (300,000 square kilometers), constituting two percent of the total land area of the world. The country's 7,107 islands and islets are clustered into three groups: Luzon, Visayas, and Mindanao. Of these, Luzon and Mindanao comprise the two largest islands with land areas of 105,000 and 95,000 square kilometers, respectively. Together, they represent two-thirds of the total land area of the country. The archipelagic character has given the country extensive territorial waters (679,800 square kilometers) and the longest discontinuous coastline in the world. Ancestral domains cover a total area of at least two million hectares. The area covered could be larger since most indigenous communities inhabit the forest zones, which account for about 15 million hectares or half of the country's total land area.

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The country is part of the Southeast Asian region, and about 1,000 kilometers from the southeast coast of the mainland of Asia lying on the western margin of the Pacific Ocean. Its boundaries are formed by three bodies of water: on the west and north by the South China Sea, on the east by the Pacific Ocean; on the south by the Celebes Sea and the coastal waters of Borneo. The country's location makes it strategically important not only as meeting ground for various cultures but also as a distribution center of goods which include seeds in the region. Its abundant natural resources coupled with its location have attracted traders, explorers and adventurers for centuries. The legacy of these early visitors remains today, and manifested in the language, culture, ethnology, religion, architecture, and cultivated crops.

The climate of the Philippines is tropical and maritime. It is characterized by relatively high temperature, high humidity, and abundant rainfall. The mean temperature is between 25 to 27°C with a range of 21 to 34°C. The average monthly relative humidity varies between 71% in March and 85% in September. The country has two marked seasons, dry and wet on the western shores facing the South China Sea, where the dry season generally begins in December and ends in May, with the wet season covering the rest of the year. The dry season shortens progressively eastward, and the rain is heaviest along the eastern shores facing the Pacific Ocean. From June to December, typhoons frequently pass the archipelago at an average of 19 typhoons per year.

Population

The present population exerts tremendous pressure on the resources of the country. There are now about 84 million Filipinos, and the population is growing at a rate of 2.36% or 1.7 million births a year. The population of the Philippines is unevenly distributed throughout the islands due to the geographical, historical and social forces, and to the uneven development in the various regions of the country. This distribution affects the degree of exploitation of natural resources.

Biodiversity

The Philippines plays a vital role in preserving global species because of its status as a mega-diversity country and a biodiversity hotspot. As one of the 17 mega-diversity countries, it is one of the biologically richest in the world, with about 52,177 species of plants, animals and other life forms that are unique to tits territorial boundaries. More than half of the described species in the Philippines are endemic and cannot be found elsewhere in the world. According to Ong, *et al*. (2002) every parcel of land that is converted, cultivated, or developed will likely result in the loss of unique life forms found nowhere else on the planet. It is also the second smallest country in

that list, but the combination of its complex geological origins, fragmented layout, varying exposures to shifting winds and typhoons, peculiar rainfall distribution has given rise to the existing complex mix of ecosystems and habitat types.

However, as one of the 25 biological hotspots, only about 6% of its natural vegetation remains, and 499 species are listed in the 2004 IUCN Red List of Threatened Species (DENR, 2005). Of this number, more than half of the species are plants. There is a high rate of biodiversity loss due to high commercial and social demand, and the biodiversity is under constant threat of extinction from overharvesting or overcollection, pollution, widespread land conversion, urbanization, burgeoning population, and poverty.

PGR and Philippine Agriculture

As in most of the tropics, the natural vegetation of the Philippines is highly diverse. It supports one of the world's richest floral communities. In many ways, the vegetation constitutes one of the country's greatest resources. Out of 4.5 million living species in the world, 1.4 million species of flora and fauna were given descriptive names by scientists and of these about 52,177 species have been identified in the Philippines. There are approximately 15,000 species of plants in the country composed of 8,120 species of flowering plants; 33 species are gymnosperms; 1,030 species of ferns and fern-allies; and 1,271 species of bryophytes. The International Union for the Conservation of Nature (IUCN) ranks the country as fourth in the world in terms of species diversity and endemism. More than 3,000 species in the country are utilized for food, feed, fuel, shelter, fiber, medicine, beverage and ornaments.

Philippine agriculture is mostly smallhold. However, there are large-scale plantations for the production of exportable products such as banana, pineapple, and papaya. It also has competitive advantage in trading traditional export products like coconut oil, sugar, and abaca. Although the Philippines is predominantly rural, majority of the population lives in urban areas. The agriculture-dependent households represent 40 percent of the total population, but accounts for over two-thirds of the poor. Poverty incidence among rural households is more than 50% compared with 26% among urban households.

Crop improvement and development continue to be the top priority of the different national commodity programs and these rely on plant genetic resources as initial building blocks of new and improved varieties. In the pursuit of attaining food security and agricultural self-reliance, the

importance of plant genetic resources as sources of novel genes becomes even more pronounced. Recognizing the value of plant genetic resources, efforts have been made to conserve these important resources for present and future needs. In an agricultural country like the Philippines, the performance of the economy depends ultimately on PGR, whether exotic or introduced.

Status

Management and Coordination of PGR Research

As defined by IPGRI, PGR research involves exploring, collecting, characterizing and conserving plant genetic resources.

Institutional Structure

National PGR research and undertakings in the Philippines are coordinated through three national agencies, namely the Department of Science and Technology (DOST), the Department of Agriculture (DA) and the Department of Environment and Natural Resources (DENR). Depending on the task or area of concern, such coordination is carried out by the Philippine Council for Agriculture Forestry an Natural Resources Research and Development (PCARRD) for the DOST, the Bureau of Agricultural Research (BAR) for the DA, and the Parks and Wildlife Bureau (PAWB) for the DENR.

Previously, a National Committee on Plant Genetic Resources (NCPGR) was created by DOST to oversee the National Plant Genetic Resources Program of the Philippines. The NCPGR operated from 1984 to 2000 through PCARRD, its commodity R&D teams, and various crop technical committees, with the National Plant Genetic Resource Laboratory (NPGRL) of the Institute of Plant Breeding (IPB) as secretariat. In 2001, upon the recommendation of an ad hoc committee to review NCPGR's mandate, the committee's coverage was expanded to include animal and microbial genetic resources. The NCPGR effectively filled in the gap of coordinating PGR activities even without an operational budget. Its activities were funded and coordinated by PCARRD-DOST.

Then, in 2001, the Philippine National Network for Plant Genetic Resources for Food and Agriculture (PGRFA) was established to sustainably manage and safeguard the country's PGR, through the Department of Agriculture and coordinated by the Bureau of Agricultural Research

(BAR). The Seed Industry Act of 1992 which served as the basis for the establishment of the National PGRFA Network spelled out the participation of PCARRD in its implementing rules and regulations as……"the PCARRD shall coordinate, evaluate, and monitor the research and development components of the Seed Program in line with the National Commodity R and D Programs". Part of the Seed Program relates to conservation and utilization of the plant genetic resources (Eusebio and Lopez, 2002). The PGRFA Network aimed to operationalize a national system for collecting, conservation, regeneration, multiplication, characterization, evaluation, documentation and utilization of PGR. The Network's role was to review existing programs and policies related to PGR conservation and utilization, develop and package national agenda for PGRFA and identify and substantiate the institutional capability of each member institution. The Bureau of Plant Industry under DA was the base of the PGRFA Network.

Further, at the DA, the Office of the Undersecretary for Policy and Planning coordinates activities related to policy. To accomplish the task at hand, this office taps PGR experts from various institutions on an on-call basis. The experts do their best to fulfill their assigned tasks, but the strength and validity of the output reflects the composition of the expert group.

Different institutions in the Philippines continue to make deliberate efforts to collect and maintain plant germplasm as part of their crop improvement programs; these institutions are members of the National Agriculture and Resources Research and Development Network (NARRDN). But for national initiatives on PGR related to agriculture and food, the National Plant Genetic Resources Laboratory (NPGRL) of the Institute of Plant Breeding (IPB), the University of Philippines, Los Baños (UPLB) is the national center for conservation and utilization of important and potentially useful agricultural crops. As such, NPGRL has the mandate to conserve the endemic and introduced PGR, provide plant breeding projects and programs in the national research system with a broad genetic base for crop improvement, and monitor and coordinate national effort in the collection, conservation, utilization and exchange of PGR.

PCARRD and its R&D Network

PCARRD participates actively in PGR research management, specifically in monitoring and evaluation; proposal packaging, evaluation and funding; resource generation and collaborative linkages. PCARRD is one of the sectoral councils under the Department of Science and Technology. IT is the government arm that plans, coordinates, monitors, and evaluates the national R&D directions and efforts in agriculture, forestry, and natural resources sectors. Its primary clients are member-agencies of the National Agriculture and Resources R&D Network

and the regional R&D consortia. Other key clients include policy makers, R&D workers, non-government organizations (NGO), local government units, and the private sector.

International Initiatives and Collaborative Linkages

From the perspective of international initiatives, compliance with the Convention on Biological Diversity (CBD), particularly concerns relating to the environment and biosafety, are undertaken and spearheaded by the Department of Environment and Natural Resources (DENR), through the Parks and Wildlife Bureau (PAWB). On the other hand, food and agriculture concerns of the FAO are undertaken and spearheaded by the Department of Agriculture (DA), through the Office of the Use for Policy, Planning Research and Regulation.

Collaborative arrangements are facilitated through networking. Networks provide a mechanism for sharing information/knowledge and germplasm, transferring technology and standardizing procedures, as well as in undertaking collaborative R&D programs, including capability building. The crop germplasm networks facilitate the standardization of germplasm collection, maintenance, evaluation and documentation, and also enhance capacity building that includes exchange of experts/scientists and upgrading of facilities. Networks also help delineat responsibilities and optimize the use of available financial and material resources (Escaño and Tababa, 1997).

In the Asian region, a network, like the Regional Cooperation in Southeast Asia for Plant Genetic Resources (RECSEA-PGR), provides an appropriate and excellent avenue for translating the Global Plan of Action into specific and regional concerns. RECSEA was established in 1977. It is a network envisioned to protect and harness the plant genetic resources diversity in SEA for food, nutrition and health; poverty reduction; and sustainable environment protection. Guided by the principle that collaboration among member countries in the utilization of PGR for the well-being of the region and humankind is essential, RECSEA-PGR operates in accordance with each country's legislations and commitment to international agreements on PGR. RECSEA-PGR builds and enhances the national research capacity of member countries through collaboration in and support for database development and sharing, exchange of experts, and policy advocacy. Likewise, RECSEA-PGR develops and implements a resource mobilization strategy and establishes effective linkages with regional and international organizations.

Moreover, like most countries in the region, the Philippines is a member of several networks involved in PGR collecting or other PGR-related activities. The base institutions of these networks also take part in providing services to the management of the collections. These networks and institutions are the following:

1. AVRDC - Asian Vegetable Network (AVNET), Allium Network
2. ICRISAT - Cereals and Legumes Asian Network (CLAN)
3. IPGRI/INIBAP/BAPNET – Musa Germplasm
4. CIP/UPWARD - Asian Network on Sweet Potato Genetic Resources (ANSWER)
5. IRRI - International Network for Genetic Evaluation of Rice (INGER)
6. IPGRI - International Coconut Genetic Resources Network (COGENT)

The Philippines also links with CIMMYT for corn germplasm and information exchange, and is a member of the Council of Partnership for Rice Research in Asia (CORRA) which is a joint initiative to strengthen partnership among rice research systems in Asia.

The scope of collaborative arrangements include: planning and implementation of R&D projects; capacity building through exchange of scientists, participation in scientific and technical activities of mutual interest such as symposia, trainings/workshops, conferences, R&D projects; exchange of germplasm/genetic or plant materials, scientific/technical literature, information, methodology genetic/ plant materials required for the collaborative projects.

Current and New Undertakings

Conservation in simple and practical ways had been ongoing for ages, as reflected in the way the indigenous people cultivate and protect the plants identified and selected by their forefathers for several generations.

The government's PGR conservation activities are conducted within the respective institutional mandates. Special projects in collaboration with local and foreign donors and counterpart scientists in other countries/regions augment and complement ongoing conservation efforts. There are specialized agencies that have the mandate to develop specific crop-based industries, and identified to conduct R&D functions, and they are able to focus their PGR activities through a regular program on collecting, maintenance, characterization and evaluation of their priority crops. However, the scope, extent and duration of these efforts usually depend on the availability of funding.

PGR Collecting, Characterization and Evaluation, Documentation and Utilization

Existing Philippine collections (Table 1) are conserved by 45 institutions. Majority are seeds maintained in cold storage facilities while the asexually propagated species are maintained in field genebank and through *in vitro* conservation.

Of the total PGR accessions, 90% have complete passport data. Characterization and evaluation of the accessions are still being completed; currently only 40% have been characterized morphologically, 3% using biochemical and 7%, molecular markers. Diversity assessments using micro-satellite markers (simple sequence repeats) have been conducted in coconut in field genebank and farmers' fields. NPGRL (2004) reported that 60% of the total Philippine collections were evaluated against biotic and abiotic stresses; only NPGRL has data on indigenous knowledge (20% of the collections), and on distribution (only 20-30%).

Table 1. National germplasm collection in Philippines.

Crop Group	Number of Species	Number of Accessions
Cereals	4	9,700
Food Legumes	30	15,926
Forage/pasture	7	27
Plantation/Industrial crops	55	4,890
Root Crops	13	3,969
Medicinal and Spices	98	793
Fruit trees	138	2,970
Nut trees	13	257
Ornamentals	196	3,224
Botanical collection	75	75
Vegetables	115	13,067
Unknown species		8,636
Total	**744**	**64,351**

Source: NPGRL, 2004.

Utilization of germplasm materials is basically through requests by research institutions in their R&D activities and crop improvement programs, for the development of improved varieties and the selection of outstanding genotypes. The varieties released by these programs are

ultimately used by small farmers and other resource users in the communities, both locally as well as in other Asian countries. End-users of these germplasm materials consist of students, farmers, international research institutions, and other private entities.

NPGRL

In pursuit of its functions, the NPGRL holds 644 accessions consisting of cereals, legumes, forage and pasture crops, fruit trees, legumes, plantation/industrial crops, root crops, ornamental crops, medicinal spices, and vegetables. A large proportion of this collection, estimated at 75%, is of local origin. For several crops (except rice), it is the NPGRL as national center for conservation and utilization of important and potentially useful agricultural crops, that conserves germplasm under cold storage for long- and medium-term seed conservation, characterization (morphological, cytological, biochemical, and molecular levels), documentation, and utilization, maintains *in vitro* conservation in the laboratory, and maintains field gene bank for selected crops.

The NPGRL holds an extensive collection of maize with over 2,400 accessions, 49% of which are collected locally and representing unique germplasm of the crop that are now difficult or impossible to find in the farmers' fields. A great majority of its germplasm collection of vegetables consists of indigenous species and collected from farms and other habitats all over the country, as well as vegetable germplasm from Thailand, Laos, Cambodia, Vietnam, Bangladesh, and South Korea. Majority is the world base collection center for germplasm of winged bean (*Psophocarpus tetragonolobus*), snake gourd (*Trichosanthes* spp.), bitter gourd (*Momordica charantia*), and wax gourd (*Benincasa hispida*).

NPGRL also holds the duplicate world collection of mungbean (*Vigna radi*ata) totaling 6,889 accessions, the duplicate Asian collection of tomato (*Lycopersicon lycopersicum*) with 4,751 accessions, and the country's largest collection of tropical fruits and nuts, including mango, banana, pili (*Canarium ovatum* Engl.) , and over 100 species of endemic or indigenous fruit and nut trees.

Other Institutions

Examples of institutions providing conservation of priority collections for selected crops are elucidated below:

1. Rice

The DA-PHILRICE does cold storage conservation, characterization, and evaluation of rice and maintains database for rice germplasm collection. Samples of germplasm are available through a standard material transfer agreement. Data on germplasm are available through the Germplasm Management System (GEMS). PhilRice conserves about 6,376 accessions (Eusebio and Lopez, 2002).

IRRI-GRC (Genetic Resource Center) collects, conserves, characterizes, evaluates and ensures the continued availability and exchange of rice genetic resources, and ecogeographic and agronomic data related to the germplasm in the International Rice Genebank Collection, in accordance with international conventions, agreements, and policies. IRRI distributes rice germplasm materials with conformity to all relevant national, international, and bilateral agreements and legislation (phytosanitary regulations, import/export regulations, appropriate Material Transfer Agreement (MTA). All data about the IRGC are publicly available on-line through the Internet.

The UPLB Department of Agronomy collects, characterizes, evaluates and conserves rice germplasm at short-term storage facility. It documents and makes available information on conserved materials.

Organized dissemination of improved rice germplasm and information is facilitated by the International Network for Genetic Evaluation of Rice (INGER), which makes sure that there is unrestricted, free and safe exchange of rice germplasm and the free sharing of information not only among NARES and IARC partners, but also with the private sector. There is also a joint initiative to strengthen partnership among rice research systems in Asia through the Council for Partnership on Rice Research in Asia (CORRA). The Council is the body that sets policy issues that run across the existing partnership mechanisms for rice research in Asia.

2. Banana

In the case of banana, the DA-BPI-DNCRDC maintains germplasm collection of banana, Conserves banana germplasm *in vitro* (slow growth), and maintains database for banana germplasm collection (MGIS).

Valmayor *et al. (2002)* accounted for 91 cultivars of wild and cultivated species classified according to the three-tier system: species, genome group, and cultivar. More than half (45) are *acuminata* clones. The hybrids, *Musa x paradisiaca* follow in importance with 29 varieties while 14 pure *balbisiana* are recognized. One *Musa fehi* clone and 2 unidentified accessions complete

the national banana variety collection lists. According to type and usage, dessert bananas are the most common with 47 cultivars. The cooking bananas with 34 clones are second in importance while 7 are classified as dual purpose, consumed either fresh or cooked.

Collaborative arrangements are made with the International Network for the Improvement of Banana and Plantain (INIBAP) of the International Plant Genetic Resources Institute (IPGRI) and the Southeast Asia Banana Germplasm Resources Center (SABGRC) based in the Bureau of Plant Industry – Davao National Crop R&D Center, Philippines and national germplasm centers maintaining banana germplasm in the region. INIBAP coordinates a global research effort on *Musa*, promotes and strengthens research collaboration in national and global levels, while SABGRC retrieves and collects all banana cultivars within the Southeast Asian region.

Strong collaboration with INIBAP has strengthened the country's banana germplasm conservation and utilization activities and consequently contributed significantly to the improvement of existing banana cultivars. INIBAP was instrumental in the maintenance of a regional banana germplasm in the country located at Davao, Philippines under the supervision of the Bureau of Plant Industry-Davao National Crop Research and Development Center. This Center serves as repository of important banana cultivars and accessions coming from different Southeast Asian countries. The Center maintains the banana field genebank using suckers as materials for replants. Eusebio and Lopez (2002) reported that there is a total of 205 accessions consisting of 88 from the Philippines; 45 from Papua New Guinea; 17 from Thailand; 5 from Indonesia; 22 from Malaysia; 32 ITC accessions and 4 reference materials are planted. For *in-vitro* genebank, a total of 147 accessions consisting of 64 from the Philippines; 33 from Papua New Guinea; 13, Thailand; 8, Indonesia; 18, Malaysia; 2 ITC accessions and 9 from IMTP Sigatoka Trial Phase II were reestablished.

Regional collaboration is enhanced through the Banana Asia Pacific Network (BAPNET), as INIBAP facilitates BAPNET activities in the following areas: germplasm management, information development and exchange, and strategic planning. Thus, regional priorities are established and reviewed regularly by the BAPNET secretariat. INIBAP also upgrades the capability of scientists/researchers and banana growers through trainings, particularly on the production and utilization aspects.

3. Root crops

The Philippine Root Crops Research and Training Center (PRCRTC) at Leyte State University (formerly VisCA) and the BSU-NPRCRTC have regular programs on collection, maintenance, characterization and evaluation for sweet potato, cassava, taro, and other root

crops. For instance, in the case of sweetpotato, LSU-PRCRTC and the BSU-NPRCRTC conserve germplasm in vitro (slow growth) and in field gene bank (FGB). Non-government organizations also initiated community-based projects to conserve PGR *in situ*.

There are 850 sweet potato accessions maintained in pots and 925 accessions maintained in plots. For cassava, 301 accessions maintained in the field composed of local, introduced and elite clones from the advance selection of materials from CIAT (Centro International de Agricultura Tropical) in Colombia. All accessions have been characterized based on IPGRI's descriptor list. For taro, 312 accessions are now being maintained in the field. The collection was substantially boosted by introduced varieties acquired from Indonesia, Malaysia, Thailand, Vietnam, and Vanuatu. For other root crops, PRCRTC has 35 arrowroot and 392 yam collections (Eusebio and Lopez, 2002).

Collaborative arrangements are made with the International Potato Center (CIP) through the Asian Network for sweet potato Genetic Resources (ANSWER). CIP supports germplasm conservation at national and global levels by monitoring duplicate collections, supplying clones as potential parent material for national breeding, providing training and expertise support in germplasm characterization. Sweet potato is one of CIP's mandate crops through which it seek to achieve food security and reduce poverty through scientific research and related activities. On the other hand, ANSWER employs various strategies (e.g. *ex situ, in vitro*, cryo-preservation) for the conservation of sweet potato genetic resources. ANSWER has also initiated capacity building among member-countries with regard to maintenance, characterization, evaluation and documentation of their respective sweet potato genetic resources.

4. Coconut

The DA-PCA maintains germplasm collection of coconut and does pollen and slow growth conservation of coconut and maintains database for coconut germplasm collection. The country holds the world's largest collections, now with a total of 256 accessions consisting of 106 talls, 52 dwarfs, and 98 hybrid lines (Rivera, 2006 personal communication). The LSU-NCRC and UPLB-Department of Horticulture maintain some coconut germplasm in relation to their crop improvement projects.

PCA reported that the agency's coconut accessions, including duplicates, are listed in the International Coconut Genetic Resources Database (CGRD) established by IPGRI-COGENT. The Philippine Coconut Authority has an ongoing project with IPGRI-COGENT on "Collection and evaluation of coconut cultivars and conservation of genetic resources" which enabled it to assemble the world's largest coconut collection.

The International Coconut Genetic Resources Network (COGENT) of IPGRI has a regional network in Southeast Asia. The coconut accessions of Southeast Asia are listed in the Coconut Genetic Resources Database (CGRD) established by IPGRI-COGENT.

A project on "*Poverty reduction in coconut growing communities"* in collaboration with IPGRI and other Asian countries, found that involving landowners in communities where most farmers are tenants is beneficial in promoting the diversity of coconuts and other crops. In most project sites, availability of space and tenurial status of farmers and their perceived benefits were major factors that dictated the extent of planting coconut seedlings, particularly the number of seedlings to be planted.

5. Sugarcane

Conservation of this crop involves a public-private sector partnership where germplasm collection is maintained both in a public specialized agency, the Sugar Regulatory Agency (SRA) and a newly created private institution tasked to pursue R&D activities in sugarcane, the Philippine Sugar Research Institute (PHILSURIN). The SRA-La Granja Research Center has a total of 1,244 collections with 590, foreign; 409, local; and 6 noble canes. On the other hand, PHILSURIN – Victorias Milling Corporation has about 1,000 germplasm collections, 50% of which are foreign.

Special Projects

Government institutions augment and complement ongoing conservation efforts through special projects funded by other government agencies and/or in collaboration with the private sector, growers, industry stakeholders or foreign counterpart institutions. Such projects contribute immensely to the genetic resources collection, as well as in their characterization, evaluation and utilization.

In addition to the projects briefly mentioned earlier, other examples of special projects undertaken for conservation and development are the following:

Investigation into the conservation of biodiversity of indigenous orchids using seeds – this project collected and maintained 2,454 genotypes of indigenous orchids belonging to at least 181 taxa. In addition, a system of conservation of Philippine indigenous orchids using seeds has been developed adding new knowledge in orchid conservation.

Varietal development of papaya – 223 papaya germplasm materials were collected and used as sources of desired genes. The collections include 184 accessions from the different

regions of the country, and 39 accessions from Australia, Thailand, Malaysia, Sri Lanka, USA (Hawaii), and Indonesia Eusebio and Lopez, 2002). The genetic resources serve as gene pool wherein genes carrying desirable traits are utilized to increase yield, to improve quality, and to address specific problems such as adverse environments (saline, acidic, low fertility, high incidence of pest(s).

The two abovementioned projects received support from the Department of Science and Technology-PCARRD.

Development of advanced technologies for germplasm conservation of tropical fruit species – funded by ACIAR, this project enables IPB to develop protocols for cryo-preservation and alternative conservation and regeneration strategies, and testing of genetic fidelity of conserved materials.

Conservation and use of tropical fruit species diversity in the Philippines – jointly funded by IPGRI and DA-BAR, the project systematically collects, documents new and existing collections, provides improved guidelines for their management, and characterizes and evaluates the germplasm so that useful traits as well as valuable accessions are identified. The crops covered by the project include jackfruit and other *Artocarpus* species, pili (*Canarium ovatum*), mangosteen and other *Garcinia* species, and durian (*Durio zibethinus*). Collecting and exploratory expeditions resulted in the collection of 33 new durian accessions; likewise, 40 different tropical fruit species were found during a qualitative survey, 11 fruit species were evaluated using the descriptors for tropical fruits, and 20 pili accessions in Albay were characterized.

Enhancing adoption of ICRISAT legume varieties and technologies in the Philippines – in collaboration with various state universities and colleges and line agencies of the Department of Agriculture, the project aims to enhance the adoption of ICRISAT-bred peanut, pigeon pea, and sweet sorghum; introduce and fine-tune the associated technologies; and thereby also strengthen local regional capacity and collaboration.

Safeguarding and preservation of the rice genepool – a joint undertaking of IRRI and PhilRice which focuses on collection and *ex-situ* conservation of the wild and cultivated species and strengthening of germplasm conservation. The project is able to assemble 832 traditional rice varieties, and two wild rice species *Oryza meyeriana* and *O. officinalis* are being maintained at PhilRice – Los Baños (Eusebio and Lopez, 2002).

Moreover, PCARRD-DOST facilitated three new initiatives related to PGR conservation to commence in 2007. These projects are:

Collection, conservation, regeneration and re-introduction of indigenous orchids in selected protected natural habitats – Applying the orchid germplasm conservation method previously developed in a PCARRD-funded project, "Investigation into the conservation of biodiversity of indigenous orchids using seeds", this project will collect and conserve seeds of indigenous orchids, concentrating on species in the Philippines cited by IUCN as critically endangered, endangered or vulnerable, or listed in the CITES database, to be used for additional source of regenerated plants. These will be regenerated into planting materials using *in vitro* protocols; and the plants will be re-introduced to selected protected habitats and home gardens in strategic areas of the country. Funded by DOST-PCARRD, this new project will reinforce the concept of conservation by enhancing the utilization of the indigenous orchid germplasm collection.

The re-introduction of indigenous orchids to home gardens, a novel concept in Philippine PGR conservation and management, would initiate the commercialization of indigenous orchids through family level operations. Likewise, it is expected that gathering of orchids from the wild would be reduced, as there will be alternative sources of plants derived from the laboratory or artificial sources. In addition, the new project can also link with concerned commercial gardens at or near the vicinity of the target sites to propagate and market the indigenous orchids derived from plants obtained from the project.

The project has social and economic value by providing livelihood opportunities; scientific value by giving scientists, breeders and researchers access to plants that they could work on as possible source of genotypes for improvement of the orchids; ecological value through the conservation of the indigenous orchids, especially the species that are threatened, endangered and vulnerable; and still offers to all the opportunity to appreciate the aesthetic value of these orchids. Home garden owners, ornamental plant growers, commercial garden owners, researchers, and plant breeders stand to benefit from the output of this project.

Collection, evaluation, and utilization of exportable agricultural products – this is a collaborative project of the government with the private sector. Breeders in various state colleges and universities in the Philippines, as well as from different countries, have developed many new crop varieties in the last five years. With a new paradigm involving private stakeholders, such as Mama Sita Foundation (MSF) and Exotica, instead of government research institutes, this project would make available the best of these varieties to the local growers who intend to have these grown in large scale for export. Since the private stakeholders are the primary users themselves, they have great determination to succeed and the willingness to commit funds and other resources. This project will collect, conserve and evaluate new varieties of selected crops

developed in other countries and those indigenous to the Philippines. Initially, some of the agricultural crops that will be collected, evaluated and utilized include fruit crops such as calamansi, peach palm, jackfruit, mangosteen, tamarind, mango, and guava.

Varietal improvement of selected ornamental crops through gamma irradiation – funded by DOST, this project will develop new or improved varieties of selected ornamental plants, such as *Spathoglottis* orchids, foliage-type anthuriums and Hoyas, using gamma irradiation. This project will generate a new technology on mutation induction of the ornamental crops, conserve rare species of the endangered one, and multiply newly discovered species or varieties for commercialization.

There are also sporadic studies on ethno-botany, and participatory inventory and conservation. Ethno-botanical studies in specific provinces identify the culturally significant plants and their uses as well as the farm technologies used and socio-cultural activities or traditions practiced by the indigenous people in the locality. Participatory inventory and conservation of endemic, endangered and economically important flora and fauna in selected forests are also conducted; a recent one is a study done by Amoroso *et al* (2003) in Mindanao.

Biodiversity conservation using ecosystem or landscape approaches that aim to successfully integrate strategies for conservation and development are usually conducted in protected areas. Classic examples of these types of research are (1) the SANREM-CRSP project that used the participatory approach in looking at the interdependence of ecosystems specifically the protected forest and the agro-ecosystems that extended outward, and (2) the SEARCA Biodiversity Research Program for Development in Mindanao, with funding support from the Netherlands (Arboleda, 2006). The project introduced a new approach to biodiversity research – one that is interactive, participatory, and multi-disciplinary, and used learning-based strategies to generate knowledge on biodiversity in general.

Other examples of how plant genetic resources of an institution are utilized specifically through development or generation of varieties benefiting farmers, growers and consumers of specific crops/commodities are cited below. The accessions acquired from the genebank or laboratory are evaluated, selected and either directly recommended as variety to the farmers or used in hybridization work.

Farmers have limited direct access to seeds stored at national genebanks. Desirable genes get to them through the approved varieties that are commercially available. In 2004, the National Seed Industry Council (NSIC) approved 42 new varieties for commercial planting, including the following: corn – 16 varieties; rice – 8 varieties; three each of sweet potato and

mandarin; two each of tobacco, cassava, mango and tomato; and one variety each of potato, taro, mungbean, and pomelo. The respective characteristics of these varieties and the sources of planting materials are contained in a Seed Catalog published by the NSIC (Bureau of Plant Industry, undated). Prior to their approval, these varieties were tested in various locations for their agronomic and horticultural characteristics, and they were scientifically evaluated and documented against a given standard. A number of fruit varieties registered to the NSIC include those outstanding germplasm directly utilized as commercial cultivars. They can be grouped into (1) new varieties registered from direct utilization of the germplasm – consisting of 16 varieties from 11 fruit species, and (2) traditional and introduced varieties evaluated and registered, and now used as standard varieties by NSIC – consisting of 11 varieties from 8 fruit species (Catibog, 2006).

To ensure that approved varieties possess outstanding characteristics and adapted to farmers' conditions, the National Seed Industry Council through the Technical Secretariat and Technical Working Groups established standards and procedures before a variety gets approved for commercial release. The procedures include system of screening of entries, field testing and evaluation appropriate for the crop, gathering and analysis of data from different locations, selection of nominations, approval and release.

In addition to the public institutions involved in crop improvement, private entities also participate actively in crop development. Their activities began as importation and distribution of seeds (initially vegetable and ornamental seeds) and then later to actual breeding work. With the advent of hybrid corn and the growing acceptance of hybrids, big foreign companies entered into the picture and started to avail of the multi-location testing facility of the NSIC-TWG (Eusebio and Lopez, 2002).

Other activities on PGR documentation and database management, such as those concerning information, education and communication materials/information and communication technologies (IEC/ICT) include the preparation of a publication on "State of the art on Philippine PGR." This publication contains the status of PGR conservation and management in the country, with major inputs taken from the study and survey conducted by the NPGRL of the Institute of Plant Breeding, UPLB. Another one is the development of a database on germplasm through National Information Sharing Mechanism (NISM). The Philippine Bureau of Plant Industry leads in the development of the database. The activity is being coordinated by the FAO. According to Altoveros (2005), there are existing databases on banana, corn, coconut, rice, beans, Brassica, yam, and potato that can be accessed on-site and in some cases, through the internet.

Capability Building

To fast track training and enhance the capability of personnel involved in PGR conservation, trainings/workshops are conducted consisting of lectures and practical exercises on germplasm collecting, characterization at the morphological, cytological, chemical, and molecular levels; seed, field, and *in vitro* conservation of germplasm; regeneration protocols, documentation and information management; use of internet resources; and field visits to operational genebanks.

Pilot testing of on-line course on "International laws and policies on PGR" at U.P. Open University was started in October 2005. The course is a joint project of the University of the Philippines Open University and IPGRI. It includes 8 modules and is conducted for 16 weeks on distance mode. That is standardization of the curriculum, outline and syllabus for the introductory course on PGR conservation and management in four universities teaching the course (*UPLB, BSU, LSU, USM*).

PGR R&D Management

Reactivation of the National Committee on PGR (NCPGR). Cognizant of the importance of PGR in Philippine economy, there are ongoing efforts to reconstitute the NCPGR and continue/initiate collaborative efforts between/among three departments: Agriculture, Science and Technology, Environment and Natural Resources, and the national commodity centers and institutes, the academe, private sector and non-government organizations. This undertaking is envisioned to satisfy the felt need for a coordinative body that could unify all institutional efforts and direct the focus of PGR conservation and utilization. While there are existing programs, plans and strategies, the country has to put working mechanisms in place to effectively coordinate and manage all the efforts.

Enabling Policies and Legislations

Recognizing the value of biodiversity and the need for wise utilization and conservation, policies and legislations have been and continue to be promulgated. Such policies are also spurred by the need to protect the right of communities and nations to their own biological resources. A milestone for PGR advocacy is the recent Senate approval of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). PCARRD participated actively in this advocacy. The Treaty was approved on 28 August 2006, paving the way for the Philippines' accession to the Treaty on 11 September 2006. This marked the Philippines' entry

into the community of nations in its efforts toward the conservation and sustainable use of PGR, and the fair and equitable sharing of benefits derived from their use. Also, this opened the country's access to the Global Crop Diversity Trust fund.

Other relevant policies and laws governing the protection of biological genetic resources and their habitats are the following:

Conservation and Protection of Wildlife Resources and their Habitats (*RA 9147*) – regulates access to genetic resources.

Indigenous Peoples Right Act (*IPRA Law, RA 8371*) – protects the right of the indigenous people to exclude others in exploiting natural resources within their ancestral domain. Also there is the Recognition, Protection and Promotion of the Rights of Indigenous Cultural Communities/ Indigenous Peoples and Creating a National Commission on Indigenous Peoples (*RA 9168*).

Plant Variety Protection Act (*RA 9168*) – local communities can document various plant materials into a registry of commonly known, publicly-used PGR. This registry will protect the use of these varieties.

Establishment and Management of National Integrated Protected Areas System (*RA 7586*) – provides for establishment and management of a comprehensive system which encompasses outstandingly remarkable areas and biologically important public lands that are habitats of various species of plants and animals.

Seed Industry Act (*RA 7308* – provides for the promotion and development of the Seed industry and the creation of the National Seed Industry Council.

Traditional and Alternative Medicine Act (*TAMA, RA 8423*) – gave rise to the Philippine Institute of Traditional and Alternative Health Care to formulate policies for the protection of indigenous and natural health resources an technology from unwarranted exploitation.

In addition, quarantine laws regulate the import and export of PGR to and from the Philippines, and the National Committee on Biosafety of the Philippines (NCBP) provides the framework for enhancing cooperation among various sectors in order to improve prevention, eradication and/or control of invasive alien species.

Problems and Constraints in the Conservation and Utilization of PGR

Financial

Unlike international institutions (e.g. IRRI) which have sustained funding, continuity of personnel and even personal dedication of staff, the public institutions that are holders of germplasm collections are beset by fund constraints and fast turnover of personnel that in turn lead to more limitations and technical problems.

Technical (R&D and Capability Building)

1. Sustained maintenance of project-based collected germplasm – Germplasm collection as a component of crop improvement projects/programs is a strategy for enriching the *ex situ* collections. However, projects have definite life span, and after the project has been completed, some of these collections are lost due to lack of resources to continue maintaining them. This problem is particularly evident in crops that require *in vitro* conservation and regular field re-planting.
2. Lack of technologies/protocols for *in vitro* conservation suitable for specific crops - Field conservation requires large area and exposes important collections to risk, but land is a scarce resource in experimental stations. Hence, *in vitro* conservation is needed but protocols are not available locally. Exchange of information and technologies should be promoted among member countries. A compilation of available technologies will go a long way to answer this need.
3. Need for rationalization of collections - Resources invested for maintenance grow proportionately with the number of collections. To have a cost-effective use of resources, there is a need to minimize unnecessary duplication of genotypes that leads to increase in number of collections. Determining the important collections – what and where to collect and conserve, and how to conserve – is crucial.
4. Need for germplasm valuation, and economic value analysis and projection of existing germplasm collections – A conserved plant has social, economic, ecological and scientific value. There is a need to assess and present the value of PGR in a manner that is understandable to policymakers as they are involved in decision-making and resource allocation and to the layman or the public as they are part of the resource users. Case studies or documentation of success stories depicting how PGR have been utilized to increase productivity will also put more meaning to the conservation and utilization paradigm as truly integrated activities.

5. Developing, establishing and adopting a database management system – A national inventory exists but this is not yet available on the web. There is no national documentation system yet. About 10% of existing PGR information is available in electronic format. Some institutions use descriptors and passport data but no standard is being followed. Use of passport data is not efficient in all institutions. Moreover, a webpage for PGR needs to be developed for inclusion in the existing websites of PCARRD-DOST, UPLB-IPB, and DA. Concomitantly, training of focal persons involved in database management should keep pace with the database system used, not only locally, but also in the regional and global arena.

Management/Institutional

1. Lack of national PGR program – A national PGR program has not been formally established, but genebanks with national PGR responsibility exist. Management of PGR initiatives lacks sustainability and leadership. PCARRD is providing leadership of the PGR ad hoc committee (specifically for PGR for food and agriculture) only at the interim until the lead agency is identified.

2. Need to satisfy the manpower requirements and upgrade facilities - in a recent mini-survey, PGR experts identified the need for recruitment of additional staff, upgrading of farm equipment to improve field genebank maintenance, upgrading of greenhouse and nursery, and upgrading of in vitro facilities. In addition, stability and sustainability of conservation infrastructures in the face of *force majeure* will be given serious attention. DENR (2005) reported that approximately 27.3 % of the country is vulnerable to drought, alternating with yearly floods and typhoons, causing serious land degradation and declining land productivity. A recent case in the country dramatizes the effect of such natural calamities on PGR conservation – in October 2006, a strong typhoon ("Milenyo" – international code name "Xangsane", with a velocity of about 190 kph) hit Luzon and severely wrought damage to the NPGRL genebank building and its facilities. The two-meter high mud and floodwater damaged the service units located at the ground floor of the building, including the seed research unit, morpho-anatomical unit, the germplasm documentation unit, and the greenhouse for asexually propagated germplasm.

3. Need for integration of different conservation efforts into the development plans of local government units that host biodiversity areas – there is a need to put biodiversity conservation in general into the mainstream of community development. Local government officials must also make decisions on how to balance the need to conserve biodiversity and protect the environment with the need for national development and poverty alleviation.

4. Lack of awareness of the importance (value and use) of PGR to the economy and national security – There is a need to enhance public awareness and participation in the conservation and wise utilization of PGR.

Directions, Opportunity & Plans

Directions

PCARRD crafted the Integrated S&T Agenda for 2006–2010 that will set the direction of S & T activities and investment patterns in the next four years. The PCARRD S&T Agenda includes enhancing conservation and improvement of PGR among the priority R&D agenda to address the following thematic areas: natural resource management and sustainable development, poverty alleviation, frontier and cutting edge science, and food security.

Specific R&D areas include germplasm collecting, conservation and maintenance; varietal improvement; and development of new varieties through molecular studies and genetic engineering. These R&D areas will focus on the specific crops and products (Table 2). These crops include the following: Export fruit crops – mango, banana, papaya, pineapple, pili, durian, jackfruit, pomelo; Vegetables; Root crops – yam, sweet potato, cassava; Industrial crops – coffee, abaca, rubber; Coconut and oil palm; Ornamental plants; and Sugarcane.

Opportunities & Plans

Genebanks, the enabling policies and laws, the farmers' action on conserving and using diversity and the farm production technologies that they adopt, as well as the consumers' choices for diversity in their food provide opportunities for securing food in the future. The strategies for PGR conservation and utilization will depend on the choice of the country and responsible institution. Major considerations for decision-making are usually resources, limitations, and capabilities. Funds for genebank maintenance are very limited. Each institution has to prioritize projects, define roles and deliverables.

The decision on what crops to collect and conserve, and how to conserve them takes into consideration the national importance of the crops in terms of comparative advantage and importance of collection; vulnerability or threat of genetic erosion to *ex-situ* collection, and the need for utilization and crop improvement. To cite an opportunity for such decision-making process, was a collaborative undertaking through RECSEA-PGR, wherein prioritization was done in 2005 involving 13 crops; the crops were ranked using the following as indicators of importance:

the country is a primary or secondary center of diversity for the crop, food security (it is a staple crop), agricultural development (it is a source of livelihood), income generation (it is an export or industrial crop). That process resulted in the following prioritization: rice, mango, coconut, banana, sweet potato, cassava, yam, potato, citrus, Brassica, beans, peas, and breadfruit.

In the case of field genebanks, a long-term option for some collections is to keep all materials and not focus on traditional varieties or those collected from the field. This means including traditional varieties, breeding lines, wild relatives, and obsolete varieties.

In database development and management, whatever database will be established and used should be able to synchronize or be consistent with regional and global databases.

To address the aforementioned needs, problems and issues facing PGR conservation and utilization and take advantage of favorable opportunities, some activities will be more vigorously pursued with concerted effort of key players, stakeholders and resource users. Following are future activities cited by Catibog (2006), the Philippine Country Report to the ICPGR, DENR (2005), and those raised by PGR experts and stakeholders in recent fora:

To enhance PGR management and capability building, a strong national PGR program will be developed that would harmonize the efforts of stakeholders from public and private sectors, and a coordinated networking system will be established and promoted. Cold storage facilities will be rehabilitated and upgraded. Education and formal training of PGR workers will be expanded and improved. Information exchange regarding PGR between the agriculture and natural resources sectors will be enhanced. Standard descriptors and appropriate characterization paraphernalia, such as refractometers and color charts, will be provided to germplasm-holding institutions. Consultations with stakeholders, non-degree trainings/workshops on PGR conservation, and IEC campaign promoting PGR activities as well as the importance and value of PGR conservation to increase public awareness will be continuing activities.

Packaging of focused project proposals for funding through the Global Crop Diversity Trust and other donors will be done. Product development would add value to PGR conservation through utilization. PGR research will focus on regeneration of accessions of low viability; development and utilization of standard documentation system for germplasm collections; promotion of the utilization of outstanding genotypes from genebanks; germplasm utilization through biotechnology; identification and utilization of potential wild and endangered species; further evaluation of the germplasm collections with respect to pest and disease reactions, biotic and abiotic stresses, and product quality; re-introduction of propagated endemic and rare plants; landscape approach to investigate interactions of natural, socio-economic and cultural

components of the ecosystem (spanning upland an lowland terrestrial ecosystems) to provide holistic analysis on effects to PGR diversity; development/promotion of informal systems of PGR conservation involving individual farmers, households and select groups to complement formal institutional conservation efforts; and farmer-participatory research.

The following table is R&D agenda: enhancing conservation and improvement of PGR.

Table 2. Priority R&D agenda, crops and products relevant to PGR conservation and utilization (2006-2010).

Crop	Product	R&D Area	Thematic Area
Exportable Fruit Crops			
Mango	Fresh fruits	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Banana	Fresh fruits; varieties	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Papaya	Varieties	Varietal improvement/ germplasm conservation and management	Poverty alleviation and food security; Natural resources management and sustainable development
		Development of new varieties thru molecular studies and genetic engineering	Frontier and cutting edge science
Pineapple	Fresh fruits	Germplasm conservation and maintenance	Natural resources management and sustainable development
Pili	Varieties	Germplasm conservation and maintenance	Natural resources management and sustainable development
Durian	Fresh fruits	Germplasm conservation and maintenance	Natural resources management and sustainable development
Jackfruit	Varieties	Germplasm conservation and maintenance	Natural resources management and sustainable development
Pomelo	Fresh fruits	Germplasm conservation and maintenance	Natural resources management and sustainable development
Vegetables	Fresh vegetables	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Yam (ubi)	Planting materials	Germplasm collection, conservation and maintenance	Natural resources management and sustainable

			development
Sweet potato	Planting materials	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Cassava	Planting materials	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Coffee	Planting materials	Germplasm conservation and management	Natural resources management and sustainable development
Abaca	Varieties	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Rubber	Varieties	Germplasm conservation and maintenance	Poverty alleviation and food security
Coconut	Planting materials	Germplasm conservation and maintenance	Natural resources management and sustainable development
Oil palm	Fresh nuts	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Ornamental plants	Varieties	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development
Sugarcane	Muscovado	Germplasm collection, conservation and maintenance	Natural resources management and sustainable development

Source: PCARRD (2006).

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The Management of National Plant Genetic Resources Centre in Taiwan

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Summary

A national Plant Genetic Resources Center (PGRC) was established at Taiwan Agricultural Research Institute (TARI) in 1993. The facilities include long-term, medium-term, and short-term storage rooms with different storage conditions in which are suitable for preserving orthodox seeds. The long-term storage room has the capacity of 240,000 accessions in aluminum cans sealed under partial vacuum. Up to December 2006, collecting seeds of 31,832 accessions in the long-term and 64,118 accessions in the medium-term storage room are conserved. For the field genebank, the collection and preservation of germplasm including fruit trees, medicinal plants etc., approximately, 4,800 accessions are grown and maintained in several repositories. A total of 1,734 accessions of collecting clonal germplasm are cultured *in vitro.*

Germplasm distribution remains a major function of the PGRC. Germplasm materials stored at PGRC are available to people with academic purpose and can be applied on application via internet or intranet. The offer of seeds is free of charge and together with relative information included for referring. The varieties distributed from PGRC and transferred to research institute were approximately 1,000 accessions each year. TARI has engaged in international exchange of crop seeds and seedlings. During the past five years, the average numbers of varieties introducing or collecting from foreign countries were more than 1,000 accessions each year.

In order to modernize genebank operations, promote information exchange and international cooperation on germplasm collection, a computerized information system of PGRC was completed in 1993. It is well recognized that the establishment of information system of

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germplasm enables users and breeders to search for needed information and to choose appropriate genetic materials in use on crop improvement program. PGRC finally aim to become a regional center for tropical and subtropical crop, through international collaboration on collection.

Introduction

We all realize and agree that agricultural improvement depends on both the utilization of abundant plant genetic resources and the achievement of scientific research. Plant germplasm is indeed the basis of crop improvement, plus the genetic variations and biodiversity are the motivating forces for continuous of progress in plant breeding. In recent years, the cultivation of hybrids and fixed commercial varieties has threatened the survival of indigenous varieties and wild plants. These primitive types may have genetically high disease resistance or environmental tolerance and are considered as precious source for utilization in future. In view of the long-term needs of ecological system, the sustainable conservation and utilization of plant genetic resources have called attention of the scientists and governors in countries all over the world. Additionally, the research and development of using skills, techniques, strategies to properly manage germplasm are also of importance. It is very meaningful to the understanding and maintaining the genetic diversity and conducting the precious plant genetic materials with care and efficiency. Besides, the establishment of an efficient information system can help genebank manager to better administer germplasm activities such as collection, preservation, regeneration, distribution, and exchange.

Taiwan Island has abundant flora, especially tropical and subtropical plants because of its geographical features. In recent years, according to the high enhancement in agricultural production and a mighty advance of economic progress, the native flora has been diminished by various development projects. The genetic base of fundamental crops became narrow by rapid progress in plant breeding and the large scale adoption and frequent turnover of the improved cultivars.

Taiwan has realized the importance of sustainable conservation and utilization of plant genetic resources. During the past decades, a crop committee of germplasm conservation and multiplication system has been organizing to assist in the development of high priority technologies for improving agricultural productivity. At that time, the Plant Genetic Resources Center (PGRC) was constructed and placed at Taiwan Agricultural Research Institute (TARI) in Taichung. The PGRC was inaugurating on August 10, 1993 and initiated operations. The center is administered by TARI under the supervision and funding from government.

Status of Collections and Conservation of PGRs

- **Storage facilities and genebank management**

The PGRC of the Taiwan was established at TARI in 1993. The Center has storage facilities with long-term, medium-term, and short-term storage rooms with storage conditions of -12±2 °C and 40±3 %RH, 1±2 °C and 40±3 %RH, and 10±2 °C and 40±3 %RH, respectively. Activities at the PGRC can be divided into four parts: collection, preservation, distribution, and research on preservation and identification technologies on germplasm. The targets of collection include germplasm currently kept by Seed Units within the country, the new varieties bred by breeding programs, genetic materials acquired from international exchange, and germplasm collected by exploration teams to meet specific demands. When germplasm materials are imported, they have to be examined by the Bureau of Animal & Plant Health Inspection & Quarantine (BAPHIQ) for quarantine purposes so as to make sure it is infection-free and viable for reproduction and distribution.

For the processing of collected samples of germplasm preservation, the method of preservation is divided according to the type of germplasm which is seed or non-seed (clonal materials). For the aspect of storage of plant seeds, according to the storage tolerance of seeds to low temperature and dry environment, seeds are further divided into storable and non-storable types. These storable materials include small-seeded grain crops and vegetables. They can be stored for a long time under low-temperature and low-humidity environment and are separately stored in long, medium, and short-term store rooms in accordance to the purpose of storage.

The long-term preservation with storage conditions of -12±2 °C and 40±3% RH is provided mainly for the comprehensive base collection. Careful identification and selection processing are conducted stringently before storage. Storage capacity of the long-term room is 240,000 accessions in aluminium cans sealed under partial vacuum. Stored materials are not be used for exchange. They are only preserved for regenerating fresh seed and periodically examined for seed viability level. The main purpose of this storage is for the perpetuation of the seed stocks. Seed life expectancy of seed preserved in long-term storage at this Centre will last from 30 to 50 years.

The facility of medium-term storage with storage conditions of 1±2 °C and 40±3 % RH is provided solely for the seeds of actively used materials. This storage will accommodate 250,000 accessions or samples that can be used for distribution, exchange, multiplication, and evaluation purposes. The seeds are also supplement the base collection in long-term storage. Regular germination tests and seed regeneration are required for materials in the storage in order to maintain their viability.

The short-term storage room with storage conditions of 10±2 °C and 40±3 %RH is provided for storing working materials frequently requested or used, such as those parents and progenies of the breeding material or those with particular characteristics for research purpose are kept in this storage room. The storage capacities of the short-term rooms are 200,000 accessions.

Those classified as non-storable type of seed which can not be stored under low temperature and low humidity conditions, are certain vegetables, cocoa, longan, litchi, as well as those seeds are large and fleshly. Such seeds may be stored in low temperature ranges (0-10 °C) for short periods such as 1-5 years.

- **Number of accessions by crops/species**

Up to December, 2006, the PGRC has stored totally 67,942 accessions in its long-term, medium-term storage rooms, tissue culture rooms, or field repositories. These accessions represent more than 180 families, 696genus, and 1,058 species of plant germplasm (Table 1). The collections of seeds including 31,832 accessions in the long-term and 64,118 accessions in the medium-term storage room are conserved. For the field genebank, the collection and preservation of germplasm including fruit trees, medicinal plants etc., approximately, 4,800 accessions are grown and maintained in several repositories. A total of 1,734 accessions of collecting clonal germplasm are cultured *in vitro.*

Table 1. The summary of germplasm accessions preserved in PGRC in 2006.

Crop	Scientific name	No. of passport data	No. of character data	No. of image data
Soybean	*Glycine max*	18,940	14,876	64
Tomato	*Lycopersicon esculentum*	6,745	87	1459
Mungbean	*Vigna radiata*	6,301	9,438	51
Rice	*Oryza sativa*	5,910	15,520	84
Pepper	*Capsicum annuum*	4,301	917	118
Eggplant	*Solanum melongena*	2,740	470	320
Peanut	*Arachis hypogaea*	2,112	1,479	127
Common flax	*Linum usitatissimum*	1,811	0	3
Sugarcane	*Saccharum officinarum*	1,397	590	0
Asparagus bean	*Vigna unguiculata*	1,333	516	116
Sweet potato	*Ipomoea batatas*	1,309	596	0

Corn	*Zea mays*	997	293	13
Lettuce	*Lactuca sativa*	710	0	0
Edible amaranth	*Amaranthus tricolor*	588	0	4
Sorghum	*Sorghum bicolor*	524	492	6
Chinese cabbage	*Brassica campestris* Pekinensis group	520	0	3
Snap bean	*Phaseolus vulgaris*	505	147	32
Cucumber	*Cucumis sativus*	465	48	100
Spinach	*Spinacia oleracea*	416	0	2
Tobacco	*Nicotiana tabacum*	385	0	0
Watermelon	*Citrullus lanatus*	381	134	6
Black gram	*Vigna mungo*	309	0	145
Muskmelon	*Cucumis melo*	305	354	4
Adzuki bean	*Phaseolus angularis*	280	490	25
Luffa	*Luffa cylindrica*	270	225	4
Jute	*Corchorus capsularis*	266	0	0
Cauliflower	*Brassica oleracea Botrytis group*	262	1	0
Peach	*Prunus persica*	260	183	59
Squash	*Cucurbita moschata*	234	99	15
Banana	*Musa* spp.	232	88	254
Snow pea	*Pisum sativum*	224	79	2
Bitter melon	*Momordica charantia*	219	122	20
Mulberry	*Morus* spp.	209	329	229
Garlic	*Allium sativum*	204	214	258
Chinese mustard	*Brassica campestris* Chinensis group	202	11	0
Chinese kale	*Brassica oleracea* Alboglabra group	201	102	0
Bottle gourd	*Lagenaria siceraria*	200	136	11
Asparagus	*Asparagus officinalis*	185	0	0
Mango	*Mangifera indica*	183	22	1
Mustard	*Brassica juncea*	136	20	6
Sunflower	*Helianthus annuus*	134	125	25
Taro	*Colocasia esculenta*	132	34	0
Radish	*Raphanus sativus*	129	15	0
Buckwheat	*Fagopyrum esculentum*	128	0	0

Tea	*Camellia sinensis*	102	101	415
Others		4,792	599	381
Total		**67,942**	**48,952**	**4298**

- ***In-vitro* culture of plant germplasm**

Recent studies have made it possible to use tissue culture of plant parts such as shoot meristems, leaves, buds, tubers, etc., of vegetatively reproduced plants. Many clonal materials such as potatoes, sweet potato, yam, taro, strawberry, and some ornamental plants are cultured in artificial media. It is more space-saving of intensively managed than field plantings. Tissue culture also ensures virus-free condition and pathogen-free from plants. Moreover, these materials are also desirable for international shipment and germplasm exchange. A total of 1734 clonal materials are collected and cultured *in vitro* (Table 2). In order to save labour cost in a limited space, as well as maintain the minimum growth of plants *in vitro*, the low temperature, low light, and modified artificial medium are used in the tissue culture room in PGRC.

Table2. Accessions of clonal germplasm *in vitro* conservation in PGRC

Crops	Number of varieties
Sweet potato (*Ipomoea babatas* (L.) Lam.)	1361
Yam (*Dioscorea* spp.)	22
Taro (*Colocassia esculenta* (L.) Schott.)	40
Potato (*Solanum tuberosum* L.)	22
Banana (*Musa sapientum* L.)	123
Strawberry (*Fragaria chiloensis* Duch. var. *ananassa* Hort.)	16
Lily (*Lilium* spp.)	129
Herbs	16
Amoectochilus spp.	5
Total	1,734

- **Field genebanks**

Germplasm materials such as fruit trees, sweet potato, medicinal plants, and grasses are grown in nursery field for conservation. So far TARI has maintained seven field nursery sites at different elevations for clonal germplasm. Of two nurseries at TARI, one is used for quarantine and isolation reproduction purpose and one repository site is used for medicinal plants preservation. The aim of the repository is focusing on collection, introduction, propagation, and conservation. The total collections of fruit trees repository include 45 families, 88 genera, and about 800 accessions. In this repository system, tropical/subtropical fruits such as apple, plum, peach, pear, Chinese apricot, persimmon, grape, loquat, pimple, longan, litchi, citrus, avocado, passion fruit, banana, papaya, mango, Indiana jujube, star fruit, guava, wax apple, mulberry, and some exotic fruits were preserved in field genebank (Table 3).

Table 3. Germplasm materials of fruit trees grown in field nursery for conservation.

Repository garden	Crops of preservation	Accessions
Wanshiang high-altitude field repository	Apple, Peach, Pear	72
Loona middle-altitude field repository	Peach, Plum, Chinese apricot, Persimmon	240
Wanfeng (TARI) low-altitude repository	Grape, Loquat, Peach, Plum, Chinese apricot, Persimmon	178
Chiayi tropical & subtropical repository	Pineapple, Longan, Litchi, Citrus, Avocado, Passion fruit, Banana, Exotic fruits	596
Fengsan tropical repository	Papaya, Mango, Indiana jujube, Star fruit, Guava, Wax apple	311
Guansih citrus repository	Citrus	112
Banana Research Institute	Banana	214*
Miaoli Agricultural Research and Extension Station	Mulberry	138

* Detail referring Table 4

Table 4. Collection of banana germplasm in different genome types.

Type of genome	accessions
AA	19
AB	2
BB	1
AAA	98
AAB	36
ABB	27
AAAA	1
AABB	1
Other	22
Total	214

In order to expand the functions of fruits germplasm, we investigate the growth habit, evaluate the utilization, and establish the primary database. The main purpose is to select the species or lines which may have the economic value or can be used as stocks with resistance to diseases or pests or can be used as the substrates for hybridization. Some indigenous or local plums with a distinct aroma and sweet skin (non-astringent) are adopted to breed with commercial variety and that become the important low chilling plum genepool in the world.

Most of citrus trees in Taiwan are likely infected with virus or virus-like disease. Nursery trees propagated from the material of existing trees in orchard are likely to be infected with these pathogens. Therefore, the program of establishment of virus-free citrus stock has been conducted since 1982. A two-level propagation procedure of foundation grove and scion grove was developed to improve the clonal germplasm. The mother trees in foundation grove are generated through shoot-tip grafting, heat therapy and indexed free from the above-mentioned pathogens. Scion trees from budwood are released and propagated directly from mother trees. Mother trees and scion trees are conserved in isolated screen house. There are 22 cultivars 36 lines have been preserved in scion grove.

Among the banana germplasm, using mass screening of resistant clones in indigenous plant *Musa formosana* (Warb.) Hayata, we investigate the feasibility of using root-pruning inoculation method. The results showed that the disease incidence of 'Pei Chiao' was 87%, but 0% of *M. formosana*. Browning internal symptom was appeared in 'Pai Chaio', but not in *M. formosana*. It obviously indicated the native species of *M. formsana* has high resistance to Fusarium wilt in banana. This character of disease resistance is considered as precious sources for utilizing banana gene pool in breeding program.

- **Germplasm collection of medicinal plants**

For the indigenous and local medicinal plant collection, PGRC collaborated with other agricultural institutes conducting the collection and preservation of genetic resources of medicinal plants. These materials including 23 species of Devision Pteridophytes, one species of Devision Gymnosperms, 880 species of Devision Dicotylendons, and 114 species of Devision Monocotyledons were added to PGRC collection. Among these materials, 109 species are native species (Table 5). Some materials are waiting for further regeneration, characterization, and utilization evaluation. Three conservation sites of medicinal plants were established for long-term preservation. A low altitude garden of medicinal herbs collection was set up at TARI. The germplasm of medicinal trees is located in Guansi Experiment Station and the other one repository nursery is established at Taitung District Agricultural Research and Extension Station for the manipulation of backup system. The information system of networks of medicinal plants germplasm has established to offer the academic research and genetic resources exchange. Aperiodical workshop has been held for exchanging knowledge and experiences on collection and development of native medicinal plants.

Table 5. Collection and conservation of medicinal plants added into PGRC in 2006.

Devision	Families	Genera	Species	Native species
Pteridophytes	14	16	23	0
Gymnosperms	1	1	1	0
Dicotylendons	90	312	880	92
Monocotyledons	19	56	114	17
Total	124	385	1,018	109

- **Distributions and exchange of germplasm**

Germplasm distribution remains a major function of the PGRC. Germplasm materials stored and maintained at PGRC are not only for conservation purposes but also to support research and utilization. These genetic materials are available to all persons with academic purpose and can be applied via internet or intranet. The supply of seeds is free of charge as well as including relative information for referring. In the past five years, many accessions of germplasm were distributed and transferred to domestic researchers listed in Table 6.

Table 6. Summary of germplasm distributed and transferred to domestic researchers in the past 5 years.

Crop	2001	2002	2003	2004	2005
Food Crops	13 (381)	9 (295)	5 (82)	11 (1,069)	9 (627)
Vegetable	17 (583)	12 (393)	6 (76)	28 (336)	14 (516)
Fruit Crop	1 (1)	1 (2)	-	6 (8)	-
Special Crops	22 (28)	1 (1)	2 (22)	7 (10)	3 (139)
Forage	1 (3)	-	-	1 (1)	-
Ornamentals	2 (2)	-	-	5 (5)	4 (60)
Total	56 (998)	23 (691)	13 (180)	58 (1,429)	30 (1,342)

The number of species and subsequent accessions in parentheses

International genetic resources exchange is also an important part of the center's service. Since 1960, TARI has been actively engaged in international exchange of crop seeds and seedlings for research purpose. The germplasm collections imported from foreign countries are conserved in an isolated greenhouse or repository nursery in TARI for quarantine examination. Crop seeds that go out of the center need pass through Seed Unit for monitoring and quarantine processing. In 2005, more than 1,000 accessions of seed packets were exchanged and distributed (Table 7). The diverse germplasm collection provides materials that genotypes can be particularly used to diversify production systems and chose as a source of novel varieties for development into new crops for commercialization.

Several kinds of publications have been printed from PGRC, such as proceedings of workshop on germplasm, annual report, catalogs, bulletins of genetic resources collection and exchange etc. Some publications also provide the regulations and instructions of application on germplasm which is available for exchange.

Table 7. Summary of germplasm exchanged to foreign countries in the past 5 years.

Crop	2001	2002	2003	2004	2005
Food Crops	4 (23)	3 (3)	1 (4)	2 (499)	3 (866)
Vegetable	28 (69)	18 (35)	12 (18)	3 (271)	2 (230)
Fruit Crop	4 (11)	8 (35)	1 (1)	1 (11)	-
Special Crops	-	65 (86)	-	-	-
Forage	1 (3)	2 (176)	-	-	-
Ornamentals	2 (48)	1 (1)	-	5 (13)	1 (12)
Total	39 (154)	97 (336)	14 (23)	11 (794)	6 (1,108)

The number of species and subsequent accessions in parentheses

Status of Information Systems of PGRs

- **Information system for intranet and web-based systems**

In order to strengthen collection, conservation, and utilization of crop germplasm, plus to modernize management and operation of PGRC, a computerized information system has also been completed and called Plant Genetic Resources Information System (PGRCIS). Three main activities were set for the PGRIS including (1) setting up a database, including related germplasm information for breeders and researchers to search for needed information; (2) developing a genetic resources information network within the country and around the world to strengthen the communication of information on germplasm; (3) handling the processing and storage activities of germplasm at PGRC. In order to enhance the communication efficiency of germplasm information, an easy-to-query database and information network were developed through the “hyperlink” available on the WWW. We modified the original program and made it possible to link to other germplasm information centers around the world. This Center provides fibre-optic network system to researchers and breeders who are interested in genetic resources information and can search on the web site (http://www.npgrc.tari.gov.tw). PGRC also provides an excellent information system that can manage germplasm of storage activities. This system is linked to other germplasm informatory centers around the world. Researchers in our country may utilize the linked net to obtain information on plant genetic resources.

- **Hardware Architecture**

The frame of hardware architecture of PGRIS is consisted of ORACLE database management system and UNIX operation system which were built in the HP9000 800/G40 database server. To modernize and computerize the operations of the PGRC, all personal computers of PGRC-related laboratories are able to link to the server with a client-sever local network architecture. The system has been upgraded with band width to 2,048K BPS.

The PGRIS employs ORACLE as a relational database management system which is further divided into 12 subsystems according to operative functions. These available data of plant germplasm, including passport data, characterization data, and image data have been stored in PGRIS database. The passport data include background of distribution, donor information, common/scientific name, cultural practices, collection data, pedigree for each accession, and other notes etc. The characterization data records the characteristics of germplasm, such as agronomic traits, resistance and tolerances to stresses, chemical composition, etc. As the image data of plant germplasm can help the operators to differentiate possible duplication and help the researchers to be familiar with the germplasm rapidly. These efforts are being made by PGRIS working group to build up a complete image database relative to the plant germplasm.

- **Database Contents**

There are approximately 67,942 records of passport data, 48,952 records of characterization data, and 4,298 records of image data in the PGRIS database. The summary of the statistics on data records is shown in Table 1. The users of plant genetic resources can find detail information of donor of each material in each record of data. Since the PGRC preserves numerous plant germplasm accessions and their characteristic data of each species are quite different, we organized various crop committees to define and uniform items and related descriptor states depending on different crops.

The passport data and characterization data are provided by the breeders or collecting expeditions program. To ensure the accuracy of these data, the end-users from application have been assigned to feedback their data to PGRIS for the original breeders and donors. In order to provide a simple and useful tool to the public in country, we modified the program into English version and to establish our own germplasm database in Microsoft Window operation system. The main function of the program makes users enable to input easily and maintain efficiently the passport data as well as characterization data.

Mission and Prospects

In order to maintain the quantity and quality of seeds in genebank, regeneration and characterization of the collected materials are being both at TARI headquarters and the participating farmers and other collaborating institutes. The standardized seed regeneration is being used. Regeneration procedures are based on a standard protocol that take into consideration the breeding structure of each species in order to preserve the genetic integrity of the original population in the regenerated materials.

For strengthening the acquisition, conservation, and utilization of crop germplasm, an advisory committee of plant germplasm and biodiversity has been organized to evaluate academic research proposals and the other issues pertaining plant genetic resources for supporting research and utilization. The plan calls for an amalgamation of existing capabilities of all agricultural research institutions with TARI playing a nuclear role. Under the system, TARI coordinates and collaborates with all research institutions and university on field collection, post-entry quarantine, conservation, regeneration, documentation, dissemination, and international cooperation.

The PGRC aims to serve as the storage site for all crops. Its mission also involves the development of new techniques for seed preservation and identification, provision of germplasm information, plus international exchange. We also aspire to become an international germplasm centre for tropical and subtropical crop, through international collaboration on collection, as a special feature of this centre.

Germplasm is the foundation of agricultural production. Regardless of future development in biotechnology (e.g. gene manipulation, tissue culture, etc.), the practical use of advanced biotechniques is not possible without germplasm. With the rapid loss of germplasm materials nowadays, the gathering and preservation of germplasm take on new urgency. Although we now have good facilities and equipment, we shall need help from all concerned people to provide us with information on germplasm and materials as to expand our holdings and to raise the level of research and agricultural production through richer germplasm collections.

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Molecular Diversity and Evolution of Aromatic Rice in Thailand

By

Apichart Vanavichit[1]

Introduction

The rice world market ranks aromatic rice at the top. For the 4-5 million Tons of aromatic rice worth 2-2.5 billion US Dollars, Thai Jasmine rice shares almost half-half with Basmati rice from India and Pakistan with small players like USA, Vietnam, and recently Cambodia. The lucrative market will become more competitive in the near future from new players like USA, Australia and Vietnam that has launched new plant type that combines high grain yield with aromatic quality.

Biodiversity of Aromatic Rice

Existing aromatic rice cultivars belongs to three groups according to isozyme analysis (Khush et al, 2000). Group I includes Thai Hom Mali and some aromatic rice varieties from China, Vietnam and Cambodia. Group V includes aromatic rice varieties from indian subcontinent including Basmati. The center of origin of the Group V aromatic rice is the foothill of Himalayas in India where large variation of aromatic rice exists. From the foothill, aromatic rice spreads northwestward to as far as Myanmar (Khush et al., 2000). No member of Group V was found in the Southeast and East Asia. The last is Group VI including aromatic rice from Indonesia, the Philippines and China. Azucena and Milagrosa are two well known aromatic rice varieties from the Philippines. Aromatic rice found in Italy, France and others are believed to be introduced from Asia when European convoys reached South and Southeast Asia several hundred years ago.

In Thailand, more than 600 aromatic landraces were distributed in four ecotypes, the deep-water, lowland rainfed, irrigated and upland ecotypes. In lowland rainfed rice ecotype, most aromatic rice varieties found here is tall and photoperiod-sensitive in contrast to the

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ones found in the upland. The infamous Jasmine rice was first reported in Chacherng Sao province during the seed purification program for elite landrace. In order to determine the population structure of these invaluable germplasm, different molecular markers were used.

Molecular Marker Systems

Phenotypic markers are not an ideal for phylogenetic studies due to the limited abundance, low degree of polymorphism, influenced by environments (GxE), and low throughput. Desirable molecular markers must abide with these properties:

- Highly abundant and evenly distributed throughout the genome,
- Highly polymorphism or polymorphic information content (PIC),
- High multiplexing ratio,
- Co-dominant,
- Neutral.

In addition, researchers must consider the development cost of the molecular markers, robustness and high reproducibility, and laboratory transfer guaranteed. A large number of molecular markers is now available, each has different advantages and drawbacks. Among these, five molecular markers commonly used are RFLP (Restriction Fragment Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) and SSCP (Single Strand Conformational Polymorphism).

Molecular Markers for Grain Aroma

Genetic markers for grain aroma were developed from molecular mapping. The locus namely *fgr* was first discovered by molecular tagging of gene controlling aroma (Ahn *et al*., 1992). Near-isogenic lines were developed from introgression a candidate segment from Della, the aromatic donor. The molecular mapping located genes for grain aroma at a likelihood of 4.5 cM near RG28, a RFLP marker located on chromosome 8. In Thai Hom Mali Rice, Khoa Dawk Mali, using bulk segregant analysis (BSU), a PCR fragment, Jasmine 500, a PCR fragment generated from Random Amplified Polymorphic DNA (RAPD) in an F2 population developed from Thai Hom Mali Rice and CT9993 (Tragoonrung *et al*., 1995) was linked to grain aroma. Because of the qualitative scoring used in those studies, they were not possible to determine quantitative trait loci for the intensity of grain aroma. The quantitative trait loci (QTL) analysis was firstly reported for grain aroma in a doubled haploid population (DH) developed from Azucena, the upland fragrance rice from the Philippines and IR64

(Lorieux *et al*., 1996). In this study, sensory test and the accumulation of 2-AP were perfectly mapped on the same chromosomal region flanked by RG28 and RG1.

Positional Cloning of Grain Aroma

High resolution mapping was developed from isogenic lines differing only for grain aroma. Map-based cloning came close to only few candidate genes. Two PCR-based markers were developed flanking the genomic segment responsible for grain aroma. Several SNPs were discovered by additional comparative sequencing between a pair of ISLs. Three open reading frames (ORF) were identified by annotation and homology search. Subsequent segregation for grain aroma from a single heterozygous F11 plants were completely associated with an 8 bp deletion in the ISLs and Thai Hom Mali Rice. This specific indel located about 729 bp from the initiation codon and within an aldehyde dehydrogenase domain of the ORF. We named this ORF Os2AP. The Os2AP consists of 15 exons. Ten more SNPs including a mutation at a 5'splice site were identified in the exon 2 but no association was found with grain aroma. This 8 bp indel was used to develop PCR-based markers, designated as 'Aromarker', for efficient marker-assisted selection in rice. Transformation of Nipponbare, a non-aromatic japonica rice, to the aromatic pair clearly demonstrated that the suppression of the Os2AP gene bring out aromatic compound.

Phylogenetic Analysis of Aromatic Rice

We compared phylogenetic analysis based on AFLP, SCAR and micro-satellite (SSR) of the aromatic rice germplasm. AFLP was used to classified aromatic rice germplasm. Selective primers were screened for polymorphic information content. Two selective primer combinations selected were MS + AAC/ER + CAA and MS + CAA/ER + CAG. The genomic DNAs were restriced with EcoR1 and Tru91 and ligated with AFLP adapters. Further selective amplification was accomplished with the second primers. For SCAR, Jasmine 500 was developed from specific amplicon that consistently aligned with aromatic rice in a mapping population. SSRs as the only co-dominant markers were screened for high PIC values Cluster analysis using Jaccard's coefficient for similarity index generated phylogenetic tree for aromatic rice germplasm. The phylogenic tree (Dendrogram) was calculated using unweighted pair-group method arithmetic analysis (UPGMA).

The results from these three type II molecular markers were that aromatic rice varieties did not form a single cluster as expected. AFLP was considered the best molecular marker because it can cluster rice into several sub-clusters. In particular, APLP can differentiate

Jasmine rice into a separate cluster. It can be concluded that aromatic germplasm was highly diversified.

The Origin of the Aromatic Gene

How many aromatic genes are found in the rice genome? This is quite an interesting questions when breed rice for aroma quality. We extensively survey all aromatic rice germplasm representing different ecosystems in Thailand including Basmati and Acuzena from India and the Philippines, respectively; using the *Aromarker* we developed here. All aromatic rices from all three isozyme groups shared the same 8 bp deletion in the *Os2AP*. It is almost conclusive to state that all aromatic genes in cultivate rices are the same. Searching for the ancestor is on the way. One possibility of the ancestor is wild rice. By comparing the limited accessions of *Oryza glaberrima* and *Oryza officinalis*, no aromatic allele of *Os2AP* was detected. This means that it is possible that *O. longistaminata* and *O. breviligulate,* the ancestor of the cultivated rice in Africa *O. glaberrima*, was not the ancestor of the aromatic gene. It is likely that the common ancestor aromatic gene could be found in *O.nivara* and *O.rufipogon*. We surveyed about 120 accessions for *O.rufipogon* and *O.nivara* using the *Aromarker* and with surprise; we found many of them carried 8 bp deletion of the *Aromarker*. All wild rices carrying the aromatic allele of *Os2AP* produced aromatic grains. We also found that many of them are heterozygous. It is now conclusive to state that the common ancestor of aromatic gene can be found in *O.rufipogon,* the wild perenial ancestor of *O.nivara*. In order to answer if it is one or many populations of wild relatives, genomic sequence of this gene must be compared.

Combining the AFLP results with the functional marker of aromatic gene, it is possible that the 8 bp deletion was naturally mutated in wild progenitors such as *Oryza nivara* and *O. rufipogon*. As the genomic sequence of most aromatic wild species and landraces are completed, we can be able to determine the origin of the jasmine rice.

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Conservation and Utilization on Vegetable, Flower, and Fruit Genetic Resources in the South of Vietnam

By

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Summary

Southern Fruit Research Institute (SOFRI) was established in April 1994. Right from the establishment, we have started collection and conservation on fruit germplasm. A total of fruit genetic resources until December 2006 are 721 varieties/clones of about 40 families of tropical and subtropical fruit trees with 348 local and 383 foreign ones. All of them have been conserving at farms, net houses of Institutes and Universities and at farmers' orchards. Evaluation on morphological and genetic characteristics has been conducted. Many of them are valuable commercial cultivars such as nam roi pomelo, da xanh pomelo, king mandarin, hong mandarin, duong mandarin, cat Hoa Loc mango, cat chu mango, abortive seed milky yellow flesh durian, Ri6 durian, Java rambutan, nhan rambutan, xuong com vang longan, tieu da bo longan, etc. Most of the introduced fruit varieties collected from Thailand, Taiwan, Australia, France, and USA. Some promising cultivars are belonging to fruit kinds such as mango, durian, rambutan, pineapple, jackfruit...; they have been tried for adaptability in the south of Vietnam.

In 2005, department of floriculture and department of vegetable were established at SOFRI, collection and conservation on flowers and vegetables have been conducted with 85 vegetable varieties/clones of 3 families and 163 flower varieties/clones of 18 families.

Introduction

Fruit genetic resources in the south of Vietnam are very rich and diversity. In addition, many fruits have high commercial values; however, rare and wild fruit varieties are still in the

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natural populations. Selective pressure on high quality and yield fruit varieties is more and more strong, so others will be lost in the future. Thus, it is very important and necessary to collect and conserve on fruit varieties, especially on rare and wild fruit varieties. At SOFRI, the genetic resources of fruit have been collected from 1994 and of vegetable and flower from 2004 and these materials are conducted to evaluate, conserve and utilize in efficiency.

Materials and Methods

Materials:

All vegetable, flower, and fruit varieties are collected and grown in SOFRI farms and farmers' orchards. These plant germplasm materials and products are used for investigating agronomical characters, and for chemical analysis in biotechnological lab and fruit quality analysis lab.

Methodology:

- Collection: All seeds, scions, buds, shoots are collected from farms, farmers' orchards, forest, etc. The procedure of collection and method of description of fruit germplasm are following the protocol of IPGRI.

- Conservation:

The implementation of conservation on fruit germplasm includes two methods: *in-situ* and *ex-situ* conservation.

1. *In-situ* conservation: germplasm conserved at farms, farmers' orchards where fruit trees growing originally.
2. *Ex-situ* conservation: germplasm collected and conserved in other farms, net house and tissue culture room et al., or at the repository nursery in research institutes or universities.

Genrally, the germplasm of vegetables and flowers are collected and then conserved in net house and *in-vitro* by using ex-situ conservation.

- Evaluation and utilization:

1. Fruit varieties have been evaluated on fruit quality characteristics, reaction to salinity, flooding, and *Phytophthora* diseases. Fruit clones/varieties bearing high yield and good quality have been selected using for mother stock trees or becoming commercial varieties as well as using as parents in hybridization. The varieties with high tolerance to salinity, flooding, and Phytophthora disease have been used for rootstock and also as mother stocks as well.

2. In our commercial fruit varieties, one or some characteristics could be improved by inducing mutation.

3. The evaluation of utilization on fruit genetic diversity in all fruit gerplasm conservation could be conducted by using RAPD and SSR molecular.

Results and Discussions

Collection and Conservation of Fruit Germplasm

For the germplasm collection and conservation of fruits from 1994 to 2006, totally, 721 varieties/clones about 40 families including 348 local varieties and 373 foreign varieties have collected and conserved (Table 1). Most of those genetic resources have been conserved at farms of research institutes, center, universities, and farmers' orchards. Germplasms of flower and vegetables have been collected and conserved in recent years at SOFRI. So far, totally, 85 vegetable varieties/clones of 3 families and 163 flower varieties/clones of 18 families have been conserved and evaluated in farm at Tiengiang (Table 2).

Table 1. Fruit germplasm collected and conserved in farm of SOFRI from April 1994 to December 2006. (SOFRI annual report, 2006)

Kinds of fruit	Scientific name	Total	No. of local var.	Introduced varieties	
				No. of var.	Origin/sources
1. Langsat	*Lansium domesticum* Correa.	2	1	1	Thailand
2. Avocado	*Persea americana* Mill.	22	7	15	USA, Australia, Israel
3. Wild mangosteen	*Garcinla loureiri*	1	0	1	Thailand
4. Pomelo	*Citrus maxima* (Burm) Merr.	81	73	8	France, USA, Thailand, China, Malaysia
5. Grapefruit	*Citrus paradisi Macf.*	7	-	7	France, USA, Australia, India
6. Orange	*Citrus sinensis* Osbeck.	51	16	35	France, USA, China, Australia, India, Japan.
7. Lemon, lime	*Citrus limon* (L.) Burm. and *C. aurantifolia Sw.*	15	7	8	France, USA, Australia
8. Rambutan	*Nephelium lappacum* L.	7	5	2	Malaysia, Thailand

9. Banana	*Musa* spp.	55	54	1	
10. Otaheite apple	*Spondias dulcis* Soland.	2	1	1	Thailand
11. Wild, hybrid citrus	*(a)*	20	14	6	France, USA
12. Walnut	*Castanea crenata* Sieb.et Zucc.	1	-	1	Japan
13. Pineapple	*Anonas comosus* (L.) Merr.	47	12	35	Taiwan, Thailand, Australia, France, Malaysia
14. Rambai	*Baccaucera* spp.	1	1	-	
15. Strawberry	*Fragaria ananassa* Duch.	1	-	1	USA
16. Peach	*Prunus persica* (L.) Batsch.	4	-	4	USA, Australia
17. Papaya	*Carica papaya L.*	28	6	22	USA, Malaysia, Thailand, Japan
18. Citrus vars. for rootstock	*(b)*	19	-	19	France, USA, Australia, India
19. Persimon	*Diospyros* spp.	8	-	8	Australia, Japan, USA
20. Kumquat	*Fortunella* spp.	6	5	1	
20. Calamondin	*Cito microcarpa mitis*				
21. Star fruit	*Averrhoa carambola* L.	18	-	18	Malaysia, Taiwan, Thailand, Israel.
22. Passion fruit	*Passiflora edulis*	2	1	1	Japan
23. Egg fruit	*Lucuma caimito* Roem.	1	-	1	Taiwan
24. Pomegranate	*Punica granatum*	2	-	2	India, China
25. Nectarine	*Prunus* spp.	8	-	8	Australia, USA
26. Water apple	*Eugenia aquea* *Syzygium* spp.	21	18	3	Thailand, Taiwan, Malaysia,
27. Plum	*Prunus salicina*	3	-	3	Australia, USA
28. Sweetsop	*Annona squamosa* L.	3	3	-	Australia, Israel
29. Sour sop	*Annona muricata* L.	1	1	-	
30. Mangosteen	*Garcinia mangostana* L.	1	1	-	
31. Tamarind	*Taramindus indica* L.	14	-	14	Thailand
32. Jack fruit	*Artocarpus heterophyllus* Lamk.	12	5	7	Thailand
33. Apricot	*Prunus armeniaca* L.	1	-	1	USA
34. Longan	*Dimocarpus longan* Lour.	20	19	1	Thailand
35. Grape	*Vitis vinifera* L.	8	-	8	Thailand, Israel
36. Custard apple	*Annona reticula*	1	1	-	
37. Guava	*Psidium guajava L.*	27	12	15	India, Malaysia, Thailand

38. Mandarin	*(c)*	43	14	29	France, USA, China, Australia, India, Japan.
39. Salacca	*Salacca edulis* Reinw.	2	-	2	Thailand
40. Sapodilla	*Manikara zapota* (L.) Van Royan.	5	5	-	
41. Durian	*Durio zibethinus Murr.*	14	5	9	Thailand, Malaysia, Australia
42. Apple	*Zizyphus mauritiana* Lamk.	2	-	2	Thailand
43. Acerola	*Malpighia glabra*	3	2	1	Thailand
44. Dragon fruit	*Hylocereus undulatus* Britte.	19	4	15	Colombia, Taiwan, France, Thailand
45. Gandaria	*Bouea oppositifolia* Meis.	2	1	1	Thailand
46. Star apple	*Chrysophllum caimito*	8	8	-	
47. Mango	*Mangifera* spp	99	45	54	Thailand, USA, Australia, Israel, Malaysia, China, India, Taiwan, Philippines.
48. Feijoa	*Acca sellowiana*	2		2	Israel
49. Miracle plant	-	1		1	Israel
	Total	**721**	**348**	**373**	

Notes: *(a): C. medica L., Limonia acidissima, Murray paniculata, Tangor (C. reticulata Blanco x C. sinensis Osbeck), Tangelo (C. reticulata x C. paradisi);*

(b): C. limon (L.) Burm., *C. sinensis (L.)* Osbeck, Citrumelo (C. *paradisi Macf.* x P. *trifoliata Raf.), Citrange (C.sinensis Osbeck.* x P. *trifoliata Raf.), C. aurantium L.,C. tachibana Tan., C. madurensis Lour;*

(c): C. reticulata Blanco, C. reticulata var austera, C. unshiu Marc;

Table 2. Germplasm collection and conservation of vegetables and flower in farm of SOFRI. (SOFRI annual report, 2006)

Vvegetables/ flowers	Science name	Family	No. of var.	Origin/Source
1. Cucumber	*Cucumis sativus* L.	Cucurbitaceae	6	Vietnam
2. Tomato	*Lycopersicon esculentum Mill*	Solanaceae	52	Vietnam (2), India (32), AVRDC (18)
3. Eggplant	*Solanum melongena L.*	Solanaceae	9	Vietnam (5), India (2), AVRDC (2)
4. Hot pepper	*Capsicum annum* L.	Solanaceae	11	Vietnam
5. Okra	*Hibicus esculentus*	Malvaceae	7	Vietnam, China
1. Gerbera	*Gerbera* sp.	Asteraceae	40	Da Lat, Tien Giang, Dong Thap - Vietnam
2. Anthurium	*Anthurium andreanum*	Araceae	11	Da Lat, Can Tho - Vietnam
3. Phyllocactus	*Epiphyllum* sp.	Cactaceae	7	Da Lat - Vietnam
4. Chrismas cactus	*Zygocatus truncatus*	Cactaceae	10	Da Lat, Can Tho - Vietnam
5. Life-plant	*Kalanchoe blossfeldiana*	Crassulaceae	6	Tien Giang, Da Lat - Vietnam
6.Chrysanthemum	*Chrysanthemum* sp.	Asteraceae	44	Tien Giang, Da Lat, Can Tho - Vietnam
7. Daisy	*Aster amellus* sp.	Asteraceae	3	Tien Giang - Vietnam
8. Orchid	*Dendrobium* sp., *Cattleya sp., Oncidium sp.*	Orchidaceae	18	Tien Giang - Vietnam
9. Strelitzia	*Strelitzia reginae* Banks.	Strelitziaceae	2	Da Lat - Vietnam, Thailand
10. Begonia	*Begonia* sp.	Begoniaceae	2	Da Lat - Vietnam
11. Torch Ginger	*Etlingera sp.*	Zingiberaceae	3	Thailand
12. Alpinia	*Alpinia* sp.	Zingiberaceae	1	Thailand
13. Iris	*Iris* sp.	Iridaceae	1	Da Lat - Vietnam
14. Jasmine	*Camellia japoniaca*	Theaceae	1	Da Lat - Vietnam
15. Gloxinia	*Sinningia speciosa*	Gesneriaceae	7	Can Tho - Vietnam
16. African Violet	*Saintpaulia ionantha*	Gesneriaceae	3	Can Tho - Vietnam
17. Carnation	*Dianthus caryophyllus*	Caryophyllaceae	2	Tien Giang - Vietnam
18. Amaryllis	*Hippeastrum spp.*	Amaryllidaceae	2	Thailand
	Total		**248**	

Source: Department of Vegetables and Department of Floriculture – (SOFRI 2006)

Evaluation of Fruit Genetic Resources

- Evaluation on morphological and agronomic characteristics

Totally, 25 among 49 kinds of fruit trees have been evaluated on morphological and agronomic characteristics. Several important characteristics were investigated and evaluated in 446 cultivars of 25 kinds of fruit germplasms listed in Table 3.

Table 3. Important characteristics were recorded and evaluated in 446 cultivars of fruit trees at SOFRI during 1994-2006.

No.	Kinds of fruits	No. of cultivars evaluated	No. of characteristics evaluated
1	Langsat	1	8
2	Pomelo	56	13
3	Grape fruit	7	13
4	Orange	49	13
5	Lemon/lime	15	13
6	Rambutan	7	11
7	Wild and hybrid citrus	10	13
8	Pineapple	28	16
9	Papaya	16	15
10	Citrus for rootstock	7	13
11	Calamondin	6	13
12	Star fruit	18	12
13	Passion flower	1	12
14	Water apple	21	12
15	Tamarind	13	11
16	Jack fruit	3	12
17	Longan	22	16
18	Guava	4	12
19	Mandarin	42	13
20	Sapodilla	2	12
21	Durian	6	12
22	Apple	1	6
23	Acerola	1	6
24	Dragon fruit	24	11
25	Mango	86	13
	Total	**446**	

- Evaluation on tolerance to salinity and flood

Vo Huu Thoai and Nguyen Minh Chau (2002) evaluated the tolerance to salinity and flood on 18 pomelo varieties. The results indicated that some pomelo varieties have high tolerance to flooding period of 30 days. These pomelo varieties are Duong Hong Pomelo (Binh Duong), Bong Pomelo (Hue), Hong Duong Pomelo (Can Tho), and Oi Pomelo (Binhduong). Pomelo varieties such as Duong Hong (Binh Duong), Hong Duong (Can Tho), Chua Pomelo (CanTho), Chua Pomelo (Ben Tre) also showed bearing high tolerance to salinity.

- Evaluation on tolenrant to *Phytophthora citrophthora* on citrus

Barley (2001) showed that 42 citrus varieties from Vietnam were tested having reaction to *P. citrophthora*. However, the results also showed that Xiem mandarin, Hong Kim mandarin, Ta mandarin, Tau orange expressed tolerance to *P. citrophthora* growing in pot condition.

- Evaluation genetic diversity on citrus and pineapple

131 local citrus varieties/clones were used for analysis on genetic diversity by using micro-satellite molecular markers (Tran Thi Oanh Yen *et al*.). 30 varieties/clones including Queen and Cayenne of local and introduced varieties were used for analysis on genetic diversity by using RAPD molecular markers (Tran Thi My Hanh *et al*., 2005).

- Fruit varieties in farmers' orchards selected for developing commercial varieties:

Many fruit varieties were selected in farmers' orchards, farms and certificated by Ministry of Agriculture and Rural Development (MARD) and then released as commercial variety, such as cat Hoa Loc mango, cat chu mango, seedless milk yellow flesh durian, mangosteen, da xanh pomelo, nam roi pomelo, duong la cam pomelo, nghe jackfruit, etc.

- Improvement of commercial fruit varieties:

Generally, commercial fruit varieties have good quality; somehow, there are one or some characteristics necessary to be improved such as the number of seeds in citrus varieties, thin skin in cat Hoa Loc mango, and fruit dropping in xuong com vang longan. The fruit breeding program inducing mutation, traditional breeding, and embryo culture were conducted in SOFRI.

The obvious results were obtained on hybridization between commercial dragon fruit variety and introduced dragon fruit. The red flesh dragon fruit cultivar – Long Dinh 1 having good quality and high yield were denominated and released.

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International Training-Workshop (2007)
The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resource
144-148

International Training Workshop on
The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources

SOFRI, Tien Giang, Viet Nam
January 9-10, 2007

Summary of Discussions

Questions/Issues Raised	Response/Suggestions
1. Commercial crops are susceptible to diseases; therefore management of these crops is quite difficult. What would be the best approach?	• Consider the possibility of small island conservation. • *In-situ* conservation is also recommended.
2. There are 150 citrus varieties in the Mekong Delta. What are the future directions for detection of fruit diseases/pests aside from those presented?	• There is no resistant cultivar at the moment • Ecological studies are important to determine the dynamics of the pest/disease. • Consider also transgenic work or gene transplant where gene resistance in an identified crop may be transferred to the priority crop.
3. Myanmar has severe diseases in orange. What is the best approach to protect these plants?	Use chemical control but it is important to spray or apply the pesticide at the critical period, because different ecosystems require different approaches.
4. Does Vietnam give incentives to small communities involved in *in-situ* conservation?	Institutional support is given to the communities through technical assistance, e.g. training on conservation, training on processing, diversity fairs, and plant variety selections. These activities will help farmers improve their livelihood.
5. How do you convince the private sector to continue conservation of important crops?	Practice on-farm conservation. Conserve crops in the field or in home gardens. For crop conservation in the field, select the crops with economic value to the community or locality and to the farmer himself. On the other hand, conservation in home gardens would best apply to crops that are important for the farmer's subsistence or livelihood.
6. Indonesia's study on	A study in Japan showed the transmission of HLB from

Huanglongbing (HLB) disease showed transmission of the disease from citrus to *Murraya*, but not vice-versa. So, at the moment, the transmission to *Murraya* may not be significant yet.	citrus to *Murraya* and likewise from *Murraya* to citrus. Therefore, this must be taken with precaution and serious concern, especially since *Murraya* is used as an ornamental plant and its trade and transport could possibly also distribute or transfer the pathogen from one place to another.

Conclusions and Recommendations

This International Training-Workshop on the Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources successfully attained its three primary objectives, namely: to provide a venue for information and knowledge sharing, to foster or strengthen multilateral and friendly relations among country representatives, and to develop new techniques or recommend appropriate strategies for plant germplasm management.

This workshop provided an appropriate venue for sharing information and knowledge on PGR through the resources reports presented by Vietnam and Taiwan, and through the country papers presented by Indonesia, Myanmar, Malaysia, Taiwan, the Philippines, Thailand and Vietnam. Specifically, the participants learned from each other about the different strategies used to manage the conservation and utilization of specific crops that are of economic importance to the countries in the Asian region. Likewise, specific projects, such as the evaluation of Myanmar rice germplasm for resistance to bacterial blight, the evolution of aromatic rice, the transmission of Huanglongbing, and the conservation of banana germplasm in Indonesia provided some details on the results of scientific studies on rice, citrus and banana germplasm.

In addition, the workshop provided a good opportunity to foster/strengthen multilateral relations. PGR experts who had met before in another training or conference were able to update each other on their present work, while most participants whop were meeting each other for the first time found new contacts. Such friendly relations were established not only through the exchange of information during the discussions, but also during the trip to the orchards and gene banks, as well as during informal talks or conversations at mealtime.

Lastly, the group had the chance to discuss and agree on some possible courses of action or initial activities that would serve as springboard for future interactions between and among the

countries represented in the workshop. The following were the suggestions and recommendations pertaining to R&D, capability building, and management/ coordination:

A. R&D

1. Develop a proposal for a collaborative project focusing on tropical fruits (e.g. pummelo, mango) and comparing the various collections in each country, using molecular markers and fingerprinting, or other modern/biotechnological methods.

2. Conduct research on cryopreservation of priority crops.
   This proposed research will involve countries that have institutions with the capability for cryopreservation in terms of facilities, manpower, and enough funds to sustain the project because cryopreservation is very expensive to sustain.

3. Use taxonomic methods to confirm the identity of crops.
   This project is needed to confirm if a crop identified and conserved in one country but given another name in another country is in fact one and the same crop. This task will be given to taxonomists. However, it was observed that taxonomists are dwindling; hence it was suggested that more students should be encouraged to specialize in taxonomy/systematics.

4. Germplasm exchange and information exchange should be continued.
   Germplasm exchange will be pursued following international laws, inter-government agreements, and in compliance with each country's policies and legislations, e.g. quarantine/phytosanitary procedures, import/export permits, material transfer agreements, etc. Compliance with such procedures and regulations would help ensure the transfer of clean materials or germplasm free from any pathogen/organism that could carry pests and diseases. Moreover, every PGR specialist should coordinate with their quarantine offices and be aware of the crops and associated pests in the quarantine list.
   Information sharing will likewise be pursued through available websites, as indicated in some of the reports, and through requests. The participants committed to continue communicating with each other through e-mail.

B. Capacity Building

Trainings/workshops on the following topics were deemed necessary to enhance the capability of PGR specialists, considering that PGR conservation is a highly specialized field and there is fast turnover of PGR personnel:

1. Training on techniques using molecular markers and cryopreservation
2. Training on *in-vitro* propagation of fruit trees
3. Workshop on *in-vitro* conservation

Such activities would ensure that PGR specialists are kept up-to-date with new techniques, scientific findings and developments concerning PGR conservation and utilization, and enhance information exchange.

C. Management/Coordination

Each country represented in this workshop should have a focal institution to spearhead the exchange/sharing of germplasm, information and updates regarding PGR conservation and utilization. For example, in the case of Vietnam, where there are several institutions working on fruit crops, SOFRI would be the lead institution that would link with TARI and FFTC. All other countries should then submit to Dr. Sheng-chung Huang of TARI the name of their lead institution and its address/contact person/telephone and fax numbers/email address.

Due to the limited time for discussions, it was not possible to develop the details of the abovementioned suggestions and recommendations. Hence, it was agreed that all countries would submit more detailed concept proposals by e-mail to: Dr. Sheng-chung Huang of TARI (schung@wufeng.tari.gov.tw). The concept proposals should at least contain the following information:

1. Project of Activity Title
2. Duration
3. Lead Institution/Country
4. Collaborating Institutions/Countries
5. Significance of the Project/Activity
6. Objectives

7. Target Outputs
8. Methodology
9. Estimated Cost
10. Source of Fund

In closing, Dr. SC Huang thanked the participants for their active participation and cooperation in making the training/workshop a successful one. He also encouraged them to submit concept proposals and from the submissions he would see how TARI or FFTC could help source out funds. He looked forward to seeing the collaboration of various institutions and countries in the realization of the proposed projects/activities.

International Training-Workshop (2007)
The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resource
149-152

International Training Workshop on *The Conservation and Utilization of Tropical/Subtropical Plant Genetic Resources*

Name list of invited speakers and observers for attending the Training Workshop

Country	Name	e-mail & address	Title of address or contribution paper
Vietnam	Dr. Nguyen Minh Chau (one of Committee Organizers)	Director, Southern Fruit Research Institute (SOFRI), PO Box 203, My Tho, Tien Giang, Viet Nam Tel: 84-73-834699 /84-91-3-962 690 Fax: 84-73-893-122 Email: mch@hcm.vnn.vn	*Welcome Remarks*
Vietnam	Mr. Nguyen Van Khang (VIP)	Director, Service of Agriculture and Rural Development Tien Giang Province Trung Luong, My Tho City, Tien Giang Viet Nam Tel: 84-73-855667	*Congratulatory Address*
Indonesia	Prof. Siti Subandiyah (Country report)	Dept. of Plant Protection, Faculty of Agriculture, Gadjah Mada University Yogyakarta, Indonesia Email: ssubandiyah@yahoo.com	*Genetic Resources of Rutaceous Plants against the Psyllid Vector Preference and Pathogen Transmission of Huanglongbing*
Malaysia	Mr. Jamadon Bin Bahari (Country report)	Senior Research Officer, Management and Utilization of Biological Resources, Malaysian Agricultural Research and Development Institute P.O. Box 12301, 50774 Kuala Lumpur, Malaysia 62-3-89437154 e-mail: jabb@mardi.my	*Current Status of Conservation and Utilization of Tropical Plant Genetic Resources for Food and Agriculture in Malaysia*
Myanmar	Mrs. Thaingi Swe (Country report)	Deputy Supervisor, Vegetable, Fruits Research and Development Center, Myanmar Agriculture Service Fax : 095-01-667991, 666094 Email c-comp@mas.com.mm ksmyint@walla.com	*Conservation and Utilization of Genetic Resources in Myanmar*

Myanmar	Dr. Thein Lwin (Country report)	Lecturer, Department of Plant Pathology, Yezin Agricultural University, Yezin, Myanmar yau@cybertech.net.mm , yymyint@humlog.com, myiyiyi@gmail.com	*Evaluation of Myanmar Rice Germplasms for Resistance to Bacterial Blight*
Philippines	Dr. Susan Sandra Lavadia Ilao (Country report)	Supervising Science Research Specialist and Head, Program Development & Resource Generation Unit, Crops Research Division, PCARRD, Los Banos, Laguna 4030 Philippines Tel: 049-536-0014/15/20 Fax: 049-536-0016 e-mail address: susan_ilao@yahoo.com s.ilao@pcarrd.dost.gov.ph	*Conservation and Utilization of Plant Genetic Resources in the Philippines: Status and Directions*
Taiwan	Dr. Hong-Ji Su (Resources paper)	Professor, Dept of Plant Pathology, National Taiwan University Counselor, FFTC Tel: 886-2-2362-5717 e-mail: hjsu@ntu.edu.tw	*Conservation, Disease-indexing and Utilization of Pathogen-free Citrus and Banana Genetic Resources in Taiwan*
Taiwan	Dr. Sheng-chung Huang (Country report)	Director, National Genebank, Taiwan Agriculture Research Institute (TARI), No. 189 Chung-cheng Road Wufeng, Taichung, TAIWAN Tel: 886-4-23302301 ext 801 or mobile: 886-963-105082 E-mail: schuang@wufeng.tari.gov.tw	*The Management of National Plant Genetic Resources Centre in Taiwan*
Thailand	Dr. Apichart Vanavichit (Country report)	Associate Professor, Agronomy Dept., Kasetsart University, Kamphangsaen, P.O. Box7, Nakorn Pathom, 73140,Thailand Tel: 66-34-282-433-4 E-mail: vanavichit@gmail.com	*Molecular Diversity and Evolution of Aromatic Rice in Thailand*
Vietnam	Dr. Nguyen Thi Ngoc Hue (Resources paper)	Center of Plant Genetic Resouces An Khanh, Hoai Duc, Ha Tay, Viet Nam Tel: 84-34-845320 Email: huengoc8@yahoo.com	*In Situ Conservation of Plant Genetic Resources in Vietnam: Achievements and Lessons Learned*

Vietnam	Ms. Tran Thi Oanh Yen (Country report)	Researcher, Southern Fruit Research Institute (SOFRI) PO Box 203, My Tho, Tien Giang, Viet Nam Tel: 84-73-893129 / 090 8 614835 Fax: 84-73-893122 Email: tto_yen@yahoo.com	*Conservation and Utilization on Vegetable, Flower, and Fruit Genetic Resources in the South of Vietnam*
Vietnam	Mr. Đoan Nhan Ai (Observer)	Center of Application and development of fruit trees - Hue city- Thua thien province / 138 Nguyễn Phúc Sinh, Hương Long, Huế City, Viet Nam Tel : 0913425652 Email: 191256@dng.vnn.vn	Poster: *Preliminary Study for Species Diversity of Fruits in Thua Thien Hue-Vietnam*
Vietnam	Mr. Pham Ngoc Lieu (Observer)	Vice Director of SOFRI and In charge of Breeding Division Southern Fruit Research Institute (SOFRI) PO Box 203, My Tho, Tien Giang, Viet Nam Tel: 84-73-834680 / 091-3 119313 Fax: 84-73-893122 Email: pnl@hcm.vnn.vn	Posters: *1/ Some Pictures on Pummelo Varieties in the South of Vietnam* *2/ Some Pictures on Mango Varieties in the South of Vietnam*
Vietnam	Dr. Bui Xuan Khoi (Observer)	Vice Director of SOFRI and Director of SEFRC Southeast Fruit Research Center P.O. Box 10 Ba Ria Town Ba Ria - Vung Tau Province, Viet Nam Tel: 84-64-897250/ 091-3 948303 Fax: 84-64-897447 Email: sefruit@hcm.vnn.vn	Poster: *Preliminary Result of Investigation, Collection, Observation and Evaluation of Fruit Genetic Resources in the South Eastern Region of Viet Nam*
Vietnam	Mr. Nguyen Tuan Phong (MSc) (Observer)	Vice Director, Research Center of Science and Technology application Tiền Giang 139 B Nam Ky Khoi nghia street, My Tho- Tien Giang, Viet Nam	Poster: *Introduction Some Mushroom Varieties in Tien Giang,Vietnam*
Vietnam	Ms. Nguyen Thi Thanh Mai (MSc) (Observer)	Speacalist, Department of Angonomy - MARD , 12 Phùng Khắc Khoan street, District 1, HCM City, Vietnam	

Vietnam	Mr. Vo Cong Thanh (Observer)	Agonomy Faculty Can Tho University 3/2 Street, Ninh Kiều District, Cần Thơ City, Viet Nam	
Vietnam	Dr. Ha Thanh Toan (Observer)	Institute of Research and Development Biotechnology, Can Tho University 3/2 Street, Ninh Kiều District, Cần Thơ City, Viet Nam	Posters: *1/ Application Amplified Fragment Length Polymorphism (AFLD) Technique Using ABI 310 Sequence for DNA Fingerprinting Study of Mango Cultivars in Mekong Delta* *2/ Analysis of Genetic Relationships among some Citrus Varieties in Viet Nam based on Internal Transcribed Spacer Sequences*
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Taiwan	Ms. Chih-Yu, Wang (Observer)	Taiwan Agriculture Research Institute (TARI) No. 189 Chung-cheng Road Wufeng Taichung, TAIWAN Email: cywang@wufeng.tari.gov.tw	
Japan	Dr. Katsuya Ichinose (Observer)	Japan International Research Center for Agricultural Sciences (JIRCAS) 1-1, Ohwashi, Tsukuba, Ibaraki, 305-8686, Japan fourmi@hotmail.co.jp Ichinose@hcm.vnn.vn	
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